GENERAL METHOD FOR THE SYNTHESIS OF PSEUDODISACCHARIDES

Diels-Alder approach to the synthesis of pseudodisaccharides

Mohamed Hussain Haji Abdullahi

Submitted for the degree

Of Doctor of Philosophy

Institute of Cancer Therapeutics

University of Bradford

ABSTRACT

This thesis describes a new method for the synthesis of pseudodisaccharides containing a carbasugar analogue attached to a “true” sugar. The methodology is based on a Diels-Alder cycloaddition of vinyl sugars and appropriately substituted pyran-2-ones, followed by chemical manipulation of the resulting cycloadducts. The thesis also describes the synthesis of inhibitors of Golgi α-mannosidase II and glucokinase.

The first chapter is a comprehensive survey of the reported synthetic routes to pseudodisaccharides from the literature.

The results and discussions are presented in chapter 2. This chapter starts by discussion of the preparation of vinyl sugars and pyran-2-ones and the regio- and stereoselectivity of their cycloadditions. This is followed by reporting the chemical manipulations of these cycloadducts and the synthesis of a pseudodisaccharide. Cycloadducts are shown to lose carbon dioxide at elevated temperatures to afford dihydrobenzenes. The loss of the bridging carbon dioxide from the cycloadducts is experimentally and computationally investigated. The resulting dihydrobenzenes are shown to also be useful as precursors in the synthesis of pseudodisaccharides. The chemical manipulation of these dihydrobenzenes is used towards the synthesis of a pseudodisaccharide.

The third and fourth chapters focus on the synthesis of new inhibitors of Golgi α-mannosidase II and glucokinase respectively. A range of 6-aminoglucose and mannose derivatives were prepared and tested for the inhibition of Jack bean α-mannosidase, but were found to lack any inhibition. Similarly, a range of 6-triazologlucose derivatives were prepared but were found to lack any cytotoxicity.

The fifth chapter contains the details of the preparation, experimental procedures and spectroscopic characterisation of the synthesised chemical compounds.

Rate calculations are reported in Appendix I and the X-ray crystallographic data are presented in the Appendix II.

ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. Kamyar Afarinkia for giving me the opportunity to work on this interesting project and for his guidance and support throughout my PhD.

I also thank my family, especially my parents for their love, encouragement and continuous support.

I acknowledge Dr. Ian Scowen for expert X-ray crystallographic analysis, Dr. Derek Maitland for helping me with NMR data, and Andy Healey for running mass spectroscopy.

I also thank Dr. Victoria Vinader, Dr. Helen Sheldrake, Dr. Robert Falconer and Dr. Klaus Pors for their help and advice, and my colleague Martin Royappa for his support and friendship.

ABBREVIATIONS

Å	angstrom
Ac	acetyl
AM1	Austin model 1
aq.	aqueous
ADP	adenosine diphosphate
ATP	adenosine triphosphate
Bn	benzyl
Bu	butyl
BVE	butyl vinyl ether
Bz	benzoyl
cat.	catalyst
conc.	concentrated
DA	Diels-Alder
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	dichloromethane
DC-SIGN	dendritic cell-specific intercellular adhesion grabbing non-integrin
DFT	density functional theory
DIBAL-H	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DME	dimethoxyethane
DMF	*N*,*N*-dimethylformamide

DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDG	electron donating group
Et	ethyl
EWG	electron withdrawing group
FAD	flavin adenine dinucleotide
FMO	frontier molecular orbital
G6P	glucose-6-phosphate
GAPDH	glyceraldehydes-3-phosphate dehydrogenase
Glc	glucose
GlcNAc	*N*-acetyl-glucosamine
GMII	Golgi mannosidase II
GTP	guanosine-5'-triphosphate
HIF-1	hypoxia inducible transcription factor-1
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
HOMO	highest occupied molecular orbital
IC_{50}	half maximal inhibitory concentration
LDH-A	lactate dehydrogenase A
Lit.	literature
LUMO	lowest unoccupied molecular orbital
o	*ortho*
p	*para*
Man	mannose
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl

m.p.	melting point
MS	mass spectroscopy
Ms	methanesulfonyl (mesyl)
NADH	nicotinamide adenine dinucleotide (reduced form)
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PCD	programmed cell death
PDK1	pyruvate hydrogenase kinase-1
PGK	phosphoglycerate kinase
Ph	phenyl
ppm	part(s) per million
py	pyridine
RNA	ribonucleic acid
r.t.	room temperature
TBAI	tetrabutylammonium iodide
TCA	tricarboxylic acid
TBDMS	*t*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIBAL	triisobutylaluminium
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	4-methylbenzenesulfonyl (tosyl)
TS	transition state

Table of Contents

Chapter One: A Review of the General Methods for the Synthesis of Pseudodisaccharides

1.1 Introduction

In recent years, naturally occurring and synthetic carbasugars have found a great deal of application in medicinal chemistry.[1] Strictly speaking, carbasugars are defined as a class of pseudosaccharides in which the oxygen atom in the parent sugar has been replaced by a methylene group (Figure 1).[2] However, the recent literature has seen a more liberal use of the terminology and carbasugars can be more generally defined as molecules in which the sugar's polyhydroxylated furanose or pyranose ring is mimicked by a polyhydroxylated five or six membered carbocycle respectively.

Figure 1

Carbasugars lack the hemiacetal function which is responsible for a great deal of the chemistry of "true'' sugars and is the key to their oligomerisation, but mostly preserve the functional groups and shape of their parent saccharide. Since carbasugars mimic the structure of their "true'' sugar analogues, they can interact with many carbohydrate-handling enzymes, including glycosyltransferases, and with many carbohydrate-recognising receptors and proteins.[3] As these enzymes, receptors and proteins are involved in disease processes, carbasugars and their derivatives have become important tools in medicinal chemistry. Indeed, many naturally occurring carbasugars and their

derivatives have an important and wide range of biological activities hence important in the drug discovery process.[4] These include carbocyclic nucleoside aristeromycin **1**,[5] the validamycin A-H **2-9** family of antibiotics,[6] antidiabetic agent acarbose **10**,[7] trestatin B **11**, [8] and salbostatin **12**, [9] a basic non-reducing pseudodisaccharide consisting of valienamine attached to 2-amino-1,5-anydro-2-deoxyglucitol which is a potent trehalase inhibitor (Figure 2).

Furthermore, direct replacement of a "true" sugar with its "carba" analogue in an enzyme's substrate is the most straightforward means of rationally designing inhibitors of that enzyme. For example, the carbasugar analogue of cytidine monophosphate sialic acid **13**, designed as a sialyltransferase inhibitor,[10] and the carbasugar analogue of uridinediphosphate galactose **14**, designed as a galactosyltransferase and glucosylceramide inhibitor, have both been shown to inhibit their target enzymes with good potency and selectivity.[11]

Figure 2: Important molecules containing carbasugars

Within this context, pseudodi- and pseudopolysaccharides, (oligomers containing a carbasugar linked to true sugars) have a prominent role. The lack of an acetal linkage in the non-reducing end of a carbasugar means that these sugar molecules cannot be cleaved at that position by hydrolases and glycosidases (Scheme 1). Provided they are sufficiently recognised to bind at the active sites of these enzymes, they are potential enzyme inhibitors.[12]

Pseudodi- and pseudooligosaccharides have a significant advantage over the use of single residue carbasugars in that they can possess significant selectivity on the biological target. Many carbohydrate handling enzymes have a large active site that recognises a significant portion of their substrate. For example, heparanase, a β-*endo*-glucuronidase can recognise four residues spanning the cleavage site of heparan sulfate, thus giving it the ability to selectively cleave this substrate and not the plethora of heparins and other sulfated oligosaccharides.[13]

The need to have access to these biologically important molecules is becoming increasingly important. In this chapter we will explore current methodologies directed at the synthesis of pseudodisaccharides, and their biological importance. The chapter will cover the three main methodologies that have been used for the synthesis of “true” sugar-cabasugar disaccharides.

1. Coupling Reactions
2. Electrophilic cyclisation
    I. TIBAL-Induced Reductive rearrangement
    II. Titanium(IV)-Assisted Rearrangement
    III. Oxocarbenium Ion-Enol Ether Cyclisation
3. Diels-Alder cycloaddition approach

1.2 Coupling Reactions

Figure 3

The most widely used methods of making pseudodisaccharides involve the syntheses of the carbasugar as a distinct moiety, followed by its coupling to the “true” sugar. Steric and electronic factors play important roles when coupling a true sugar with a carbasugar. Joining the carbasugar at the anomeric position of the sugar is easiest, due to electronic factors (easy formation of the oxonium intermediate). Joining the

carbasugar at the primary hydroxyl is less difficult than connecting the carbasugar at the C-2, C-3 and C-4 hydroxyls due to steric factors (Figure 3). This steric problem can be overcome by employing either a true sugar epoxides or carbasugar epoxides as acceptors. Epoxides are more reactive because of the three-membered ring strain which is relieved upon epoxide opening.

Ogawa's group has contributed most in this area and their general approach is described in Scheme 2.[14] The first method involves coupling monosaccharide donors with appropriately protected carbasugar acceptors to give a pseudodisaccharide with the carbasugar located at the reducing end. The second approach employs 1,2-epoxides of 5a-carbapyranoses as 5a-carbahexopyranosyl donors.

1.2.1 Synthesis of pseudodisaccharides with a carbasugar attached to the anomeric position

Towards the total synthesis of validamycin A **2** and related compounds, Ogawa and co-workers reported the synthesis of β-D-glucopyranosylvalidamine **19a** (Scheme 3) in 1980.[15] The synthesis of **19a** assumed a structure of validamycin A which was

previously assigned by Horii and Kameda on the basis of degradation studies.[16] Condensation of a suitably protected (DL)-validamine **15** with glucose derivative **16** using mercury(II) cyanide and anhydrous calcium sulfate in a mixture of benzene and dioxane (2:1, v/v), at 65 ^{o}C for a week, gave two diastereomers α and β-D-glucopyranosylvalidamine **17a** and **17b** in 47% and 50% yield respectively. Treatment of **17a** and **17b** with 80% aqueous acetic acid afforded the corresponding dihydroxy compounds **18a** and **18b** in 44% and 76% yields, respectively. Treatment of **18a** and **18b** with boiling 10% aqueous barium hydroxide gave the desired pseudodisaccharides **19a** and **19b** (Scheme 3).[15]

Figure 4

However, on the basis of ^{1}H and ^{13}C NMR, and chromatographic behaviour, the resulting β-D-glucopyranosylvalidamine **19a** was not to be identical to the authentic sample derived from validamycin A **2**. In the same year Ogawa's group reported, using the same methodology, the synthesis of glucopyranosyvalidamine **20** (Figure 4) in which D-glucopyranose is attached to the C-1 hydroxyl group of (+)-validamine, similar to the authentic sample derived from Validamycin A **2**.[17] On the basis of these studies the correct structure of validamycin A **2** could be assigned (Figure **4**).[18]

Ogawa and co-workers also reported in 1987 the synthesis of pseudodisaccharides **25a** and **25b** (Scheme 5).[19] Protection of compound **21** with 2,2-dimethoxypropane in DMF in the presence of tosic acid afforded a mixture of 1,2:3,4-, 1,2:4,7-, and 2,3:4,7 di-*O*-isopropylidene derivatives **22a, 22b** and **22c** (Scheme 4).[19]

Condensation of compound **22a** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **16** in boiling benzene in the presence of mercury(II) cyanide and anhydrous calcium sulfate for 21 h gave diastereomeric **23a** and **23b** in 34% and 26% yields,

respectively. *O*-Deisopropylidenation of the diastereomers followed by aceylation gave **24a** and **24b** in quantitative yields (100%). Global deprotection afforded the desired products **25a** and **25b** in 72% and 70% yields, respectively (Scheme 5).[19]

Figure 5

α,α-Trehalose **26** (Figure 5) is an energy source in the plant and animal kingdoms and acts as a carbohydrate reserve in insects.[20] Trehalase, a disaccharide hydrolase, cleaves α,α-trehalose into two D-glucopyranose moieties.[21] Ogawa and co-workers have synthesised all four possible diastereomers (α,α-, α,β-, β,α-, β,β-) of 5'a-carbatrehalose **27** where one of the pyranose ring oxygens is replaced with a methylene group.[22] These diastereoisomers were synthesised by condensation of the appropriately protected pseudo-α- and -β-(DL)-glucopyranoses with D-glucopyranose derivatives in the presence of trimethylsilyl trifluoromethanesulfonate. All the pseudotrehaloses were inactive as inhibitors of the trehalase enzyme.[22]

Ogawa's group have also utilised the method described above to synthesise 5'a-carbamaltose **28**, 5'a-carbacellobiose **29** and 5'a-carbalaminarabiose **30** (Figure **6**).[23]

Figure 6

1.2.2 Synthesis of pseudodisaccharides with a carbasugar attached to non-anomeric positions

For the synthesis of pseudodisaccharides where the carbasugar is attached either at the C-3, C-4 or C-6 hydroxyl group of the true sugar component, Ogawa's group developed the use of epoxides of 5a-carbapyranoses as versatile 5a-carbahexopyranosyl donors.

Figure 7

Methyl acarviosin **31** (Figure 7) is a strong inhibitor of glycoside hydrolases. In 1996, Ogawa and co-workers reported the synthesis of methyl acarviosin derivatives and 5'a-carbamaltoses linked by amino, ether and sulfide bridges in order to study their biological activity.[24] Amino-linked 5'a-carbamaltoses **36** were synthesised by coupling

5a-carbaglucopyranosylamine **32** with methyl 3,4-anhydro-α-D-galactopyranoside **33** in a sealed tube in 2-propanol for 6 days at 120 ^{o}C to afford products **34** and **35** in 37% and 36% yields, respectively. Deprotection of **34** with methanolic sodium methoxide gave **36** in 88% yield (Scheme 6).[24]

Ether-linked 5'a-carbamaltose was synthesised by coupling epoxide **37** with the alkoxide anion derived by treatment of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **38** with NaH in DMF in the presence of 15-crown-5 ether at 70 ^{o}C to give carbadisaccharide **39** with the α-manno configuration in 45% yield. Compound **39** was acetylated and then the configuration of the C-2' was inverted by first oxidising **40** and then reducing the resulting ketone with sodium borohydride to afford **41** (45%) in the α-gluco configuration, and **39** (38%). Hydrogenation of **41** with 10% palladium on charcoal afforded the target 5'a-carbadisaccharide **42** in 76% yield (Scheme 7).[24]

For the sulphide-linked 5'a-carbomaltose synthesis, epoxide **43** was coupled with 2,3,6-tri-*O*-benzoyl-4-deoxy-4-thioacetyl-α-D-glucopyranoside **44** in HMPA and in the presence of 1,4-dithioerythritol and 2-aminoethanethiol at room temperature to afford trans-diaxially opened product **45** in 89% yield. Oxidation of **45** with DMSO/acetic anhydride followed by reduction with sodium borohydride in methanol afforded the α-gluco configuration **46** in 52% yield. *O*-Deacylation of **46** afforded the desired methyl carbamaltose **47** in 100% yield (Scheme 8).[24]

In the synthesis of unsaturated 5'a-carbadisaccharide **52**, Ogawa's group utilised *O*-benzylidenation of 5'a-carbadisaccharide **42** with benzaldehyde dimethylacetal in DMF followed by acetylation to give **48**. Refluxing **48** with NBS and barium carbonate in carbon tetrachloride afforded 6'-bromide **49** in 52% yield. Treatment of **49** with DBU in toluene afforded *exo*-alkene **50** in 90% yield. Reaction of **50** with bromine in carbon tetrachloride followed by treatment with excess sodium benzoate in DMF at 60 ^{o}C afforded alkene **51** in 45% yield. Deprotection of **51** gave the desired unsaturated carbadisaccharide **52** in 96% yield (Scheme 9).[24]

For the synthesis of sulphide-linked unsaturated pseudodisaccharide **58**, 2,3,6-tri-*O*-benzoyl-4-deoxy-4-thioacetyl-α-D-glucopyranoside **54** was coupled with epoxide **53** to afford exclusively the diaxially opened product **55** in 34% yield. Substitution of the C-7' hydroxyl of **55** with chloride is achieved by treating **55** with thionyl chloride in pyridine to give **56** in 77% yield. Dehydrochlorination of **56** with DBU in toluene afforded alkene **57** in 52% yield. Deprotection of **57** gave the desired product **58** in quantitative yield (Scheme 10).[24]

Compounds **36**, **42**, **47**, **52** and **58** were screened for inhibition of baker's yeast α-glucosidase (Table 1).[24] Only the amino-linked derivatives showed significant inhibition against the enzyme. It was concluded that amino-linked carbamaltose derivatives may be useful as inhibitors for glycohydrolases or glycotransferases, whilst ether-linked analogues were believed to be useful for the elucidation of enzymatic actions and as intermediates for the development of new active inhibitors.[24]

Table 1. Inhibitory activity of methyl 5a'-carbamaltoses **35**, **41** and **46** and unsaturated derivatives **51 & 57** against baker's yeast α-glucosidase.[24]

Compound	Inhibition at 100 μg/ml (%)
Methyl acarviosin **31**	88 (0.38)[a]
36	28
42	17
47	2
52	0
58	16

a. Numbers in parentheses denote IC_{50} (μg/ml).

To this end, Ogawa's group reported the synthesis of ether-linked 5'a-carbadisaccharide derivatives **63**, **65a** and **65b** with the α-manno configuration for the true sugar.[25] 1,2-Anhydro-3-*O*-benzyl-4,6-*O*-benzylidene-5a-carba-β-D-mannopyranose **59** was coupled with 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose **60** by heating with sodium hydride and 15-crown ether at 80 ^{o}C to afford 5'a-carbadisaccharide **61** in 52% yield. Compound **61** was then oxidised at the C-2' hydroxyl with pyridinium chlorochromate (PCC) in DCM for 5 min or with acetic anhydride/DMSO for 7 h to afford ketone **62** in quantitative yield. Reduction of **62** with sodium borohydride gave a mixture of **61** and epimeric alcohol **63**. However, reduction of **62** with L-selectride gave exclusively alcohol **63** in 77% yield. Epimerisation of **62** at C-1' was achieved by treatment with potassium *tert*-butoxide in *tert*-butyl alcohol at room temperature for 2 days to afford β-anomer **64** in 93% yield. Reduction of **64** with sodium borohydride gave a mixture of alcohols **65a** and **65b**, which after separation and acetylation gave β-manno acetate **66a** (42%) and the β-gluco acetate **66b** (25%). In contrast, reduction of **64** with L-selectride afforded preferentially the β-manno configuration **65a** in 67% yield (Scheme 11).[25]

The authors then demonstrated the versatility of these intermediates by using them for the synthesis of the carbasugar analogue of β-D-Glc*p*NAc(1→4)-D-Man*p* **71**. They did so by introducing an amine group at the C-2' position of **67**.[25] Mesylate **67** was treated with excess sodium azide in aqueous DMF at 80 ^{o}C to afford the desired azide **68** in 72% yield. Hydrogenation of **68** with Raney nickel in ethanol containing acetic

anhydride gave amine **69** in 82% yield. Treatment of **69** with a mixture of acetic anhydride/acetic acid/sulphuric acid (30:11:1) gave **70** (34%) and **71** (15%) (Scheme 14).[25]

Ogawa's group have utilised similar methodologies described above for the synthesis of ether and amino linked 5'a-carbadisaccharides **72**, **73**, **74**, and **80** as potent sialyltransferase inhibitors.[24,26,27] Sialyltransferases transfer a sialic acid residue to the non-reducing end of the oligosaccharide chain. Sialyltransferase inhibitors have attracted much attention recently[28] because sialic acid glycol-conjugates are involved in many diseases.[29] The ether-linked methyl 5'a-carba-β-lactoside **72** was prepared by coupling 1,2-anhydro-3-*O*-benzylidene-5a-carba-β-D-mannopyranoside **58** with appropriately protected sugar acceptors,[24] whereas 1,2-anhydro-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba-α-D-galactopyranose **76** was utilised to prepare the amino linked methyl 5'a-carba-β-lactoside **73**, methyl *N*-acetyl-5'a-carba-β-isolactosaminide **74**, and methyl *N*-acetyl-5'a-carba-β-lactosaminide **75** (Figure 8).[26,27] The activities of compounds **72-75** as sialyltransferase inhibitors are summarised in Table 2.[26]

Figure 8

Table 2

Compound	Inhibitory activity (IC_{50}/μM)	
	α-2,3-sialyl-transferase	α-2,6-sialyl-transferase
O-Linked 5a'C-β-Lac-OMe: **72**	419	903
N-Linked 5a'C-β-Lac-OMe: **73**	185	533
N-Linked 5a'C-β-IsolacNAc-OMe: **74**	245	265
N-Linked 5a'C-β-LacNAc-OMe: **75**	>1mM	>1mM

Bernardi and co-workers have synthesised pseudo-1,2-mannobiosides **77**, **78**, and **79** which have similar three dimensional structures and conformational behaviours to 1,2-mannobioside **80** but are more stable to hydrolysis by jack-bean mannosidase (Figure 9).[30,31] 1,2-Mannobioside **80** is recognised by the dendritic cell receptor DC-SIGN, which recognises HIV glycoprotein (gb120) and other pathogens and is therefore a potential antiviral agent.[32]

Figure 9

Diacid **81** was protected and then treated with *m*-chloroperoxybenzoic acid followed by trans-diaxial epoxide ring opening with allyl alcohol in the presence of copper triflate to afford **82**. Condensation of **82** with tetra-acetylmannose trichloroacetimidate **83** in the presence of trimethylsilyl triflate in dichloromethane at -20 ^{o}C initially afforded intermediate **84** (about 10 minutes), but leaving the reaction longer (about 20 min) the desired product **85** was obtained in 65% yield. Deacetylation of **86** with sodium methoxide in methanol gave the desired pseudo-1,2-mannobioside **77** in quantitative yield (Scheme 13).[30]

A similar approach was adapted to prepare pseudo-1,2-mannobiosides **78** and **79** starting from diacid **81**.[31]

For the synthesis of (1→6) ether-linked 5'a-carbadisaccharides, Ogawa's group coupled methyl 3-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside **86** and **36** in the presence of boron trifluoride to give protected 5'a-carbadisaccharide **87** in 37% yield. Compound **87** was converted into acetate **88** (for characterisation) and then *O*-deallyated using selenium dioxide in acetic acid/dioxane. Hydrogenation and acetylation gave **89** in 67% yield. Full deprotection of **90** using sodium methoxide afforded the desired (1→6) ether linked 5'a-carbadisaccharide **91** in quantitative yield (Scheme 14).[33]

Another route to the synthesis of (1~~1~~6) ether -linked 5'a-carbadisaccharides is the coupling of triflate derivative **91** with **92** to give protected 5'a-carbodisaccharide **93** in 86% yield. Desilylation and debenzylation followed by acetylation gave acetate **94** in 79% yield. Deacetylation of **94** afforded (1→6) 5'a-carbadisaccharide **95** in 91% yield (Scheme 15).[34]

Using similar methodologies described above, Ogawa's group have prepared 5'a-carbadisaccharide **96**[35] and 5'a-carbatrisaccharide **97**[36] (Figure 10).

Figure 10

1.3 Electrophilic cyclisation

1.3.1 TIBAL-Induced Reductive rearrangement

Sinaÿ and co-workers reported in 1997 that hex-5-enopyranoside **98** undergoes reductive rearrangement upon treatment with triisobutylaluminium (TIBAL) to afford highly functionalised cyclohexane **99** with retention of the anomeric configuration (Scheme 16).[37] The key step in this rearrangement is the *endo* cleavage of the glycosidic bond, leading to zwitterionic aluminium enolate intermediate **A**, which then recyclises and undergoes reduction to give the product **99**. These transformations complement the classical Ferrier II carbocyclisation reaction, which requires *exo*-glycosidic cleavage to release the glycan.[38] However, in contrast to the Ferrier reaction, in the TIBAL-promoted rearrangement, a glycan is retained due to the initial *endo*-glycosidic cleavage.[37]

Later, Sinaÿ and co-workers used a TIBAL-promoted rearrangement for the synthesis of (1→3), (1→4) and (1→6) ether-linked pseudodisaccharides.[39,40]

1.3.1.1 Synthesis of (1→3) ether-linked pseudodisaccharides through a TIBAL reductive rearrangement

For the synthesis of (1→4) pseudodisaccharides **108a** and **108b**, D-maltose **100** was refluxed with acetic anhydride and sodium acetate to give the peracetylated product, followed by glycosylation with thiophenol and then deacetylation to afford exclusively phenyl 1-thio-β-maltoside **101** in 68% yield from **100**. Reaction of **101** with benzaldehyde dimethyl acetal in mildly acidic DMF selectively gave benzylidene-protected thioglycoside **102** in 48% yield, followed by perbenzylation with benzyl bromide and sodium hydride in DMF to afford **103** in 55% yield (Scheme 17).[39]

Treatment of **103** with lithium aluminium hydride and aluminium chloride in DCM/diethyl ether resulted in the regioselective ring opening of the benzylidene to afford **104** in 94% yield. Trans-glycosylation of **104** with excess methanol promoted by *N*-chlorosuccinimide gave α-methyl maltoside **105** in 68% yield. Iodination of **105** with triphenylphosphine, iodine and imidazole gave iodine substituted product **106** in 93% yield. Refluxing **106** with excess DBU in THF gave the elimination product **107** in 52% yield (Scheme 17).[39]

Treatment of **107** with excess TIBAL at 50 ^{o}C promoted the reductive rearrangement to afford (1→4) ether–linked pseudodisaccharides **108a** and **108b** as a 2.5:1 mixture of diastereomers in 63% yield (Scheme 19). This moderate diastereoselectivity is rationalised by the suggestion that the hydride is delivered from the less hindered β-face of the keto intermediate.

Partially debenzylated pseudodisaccharides **109a** and **109b** were also isolated from the TIBAL reaction in 26% yield. This was the result of regioselective *O*-debenzylation at the 2-position. When the β-anomer of hex-5'-ennomaltoside **112**, derived from **110,** underwent a TIBAL assisted rearrangement, it only afforded the corresponding pseudodisaccharides **114** and **115**, and no debenzylated product was observed (Scheme 18). It was concluded that TIBAL-assisted debenzylation requires a *cis* 1,2-OR motif and that is why α-methylglycoside undergoes debenzylation whereas β-methylglycoside does not (for a rationale see Scheme 21).[39]

1.3.1.2 Synthesis of (1→6) ether-linked pseudodisaccharides through a TIBAL reductive rearrangement.

Hex-5'-enoisomaltoside **118** was synthesised using iodination/elimination reactions (see conversion of **98** to **99**) from **116**. Treatment of **118** with excess TIBAL at 50 ^{o}C gave (1→6) ether-linked pseudodisaccharide **120** in 11% yield as a single diastereomer at C-5', and 2-*O*-debenzylated pseudodisaccharide **121** in 48% yield. Also isolated from this reaction was 2-*O*-debenzylated hex-5'-enoisomaltoside **123** in 13% yield (Scheme 19).[39]

Similarly, hex-5'-enoisomaltoside **119**, derived from β-methyl isomaltoside **117**, underwent a TIBAL-promoted rearrangement and afforded exclusively (1→6) ether-linked pseudo-disaccharide **122** in 65% yield as a single diastereomer (Scheme 19). As expected no debenzylated product was observed.[39]

1.3.1.3 Synthesis of (1→3) ether-linked pseudodisaccharides through a TIBAL reductive rearrangement

In 2000, Sinaÿ demonstrated the use of TIBAL-assisted rearrangements to synthesise (1→3) ether-linked 5'a-carbadisaccharide **127** from D-maltose.[40] Disaccharide **124**, derived from D-maltose, underwent a TIBAL-promoted reductive rearrangement and an oxidation with PCC to give **125**. Treatment of the ketone with Tebbe`s reagent yielded the exocylic alkene **126** in 56% yield. Hydroboration of alkene **120** with borane-THF complex and subsequent oxidation with sodium hydroxide/hydrogen peroxide gave the desired (1→3) ether-linked 5'a-carbadisaccharide **127** in 48% yield (Scheme 20).[40]

1.3.2 Titanium(IV)-Assisted Rearrangement

Sinaÿ and co-workers have also disclosed the use of a titanium(IV)-promoted rearrangement of hex-5'-enoisomaltoside **107** to afford 5a'-carbadisaccharide **128**. Titanium(IV)-promoted rearrangement of hex-5'-enoisomaltoside **107** requires mild reaction conditions, however, it results in very low yield of the product **128** (3%) together with the major by-product bis-glycoside **129** (71%) and minor by-product, methyl 2,3,5-tri-*O*-benzyl-α-D-glucopyranoside **130** (7%) (Scheme 21).[39,41] Therefore, this rearrangement would not be a practical method for making pseudodisaccharides unless the methodology is improved significantly.

It is thought that the mechanism of the reaction involves *endo*-cleavage of the interglycosidic bond followed by intermolecular carbocyclisation to give ketone **128**.[42] It is proposed that *endo*-cleavage is reversible and the carbocyclisation step is disfavoured due to the steric demand of the aglycan monosaccharide.[43] Alternatively

complex **A** is formed which would undergo regioselective debenzylation and forms the tridentate titanium(IV) complex **B**. This would undergo either 5-*exo*-trig cyclisation to give the bis-glycoside **129** or *exo*-cleavage to give aglycon **130** (Scheme 22).[39]

1.3.3 Oxocarbenium Ion-Enol Ether Cyclisation

Mootoo and co-workers have reported a convergent methodology for the synthesis of β-carbagalactodisaccharide **131** from a central glycan component **134** and the corresponding monosaccharide alcohol **135.**[44] The key step of the synthesis is the formation of the carbasugar moiety *via* an oxocarbenium ion enol ether cyclisation. They envisaged that the esterification of carboxylic acid **134** with a monosaccharide alcohol **135**, followed by Tebbe olefination of the product would result in enol ether **133**. Activation of **133** with methyl triflate induces the carbocyclisation to form enol ether **132.** Hydroboration–oxidation of **132** would give β-carbagalactodisaccharide **131** (Scheme 23).[44,45]

The central glycan component **141** is synthesised from C-branched pyranoside **137** which is prepared from D-lyxose **136** in a four step synthesis.[46] Debenzylation of **137** using sodium in ammonia gave lactol **138**. Treatment of **138** with iodosobenzene diacetate afforded 1,2-*O*-isopropylidene acetate **139** as a mixture of stereoisomers. Treatment of **139** with thiophenol followed by basic hydrolysis and benzylation of the resulting alcohol, gave **140** as a 4:1 isomeric mixture of thioacetals in 70% overall yield from **139** (Scheme 24).

Ozonolysis of **140**, followed by the sodium chlorite oxidation of the resulting aldehyde yielded a mixture of acids **141**. DCC-mediated esterification of acid **141** with manno- and gluco-alcohol moieties **142a** and **142b** resulted in esters **143a** and **143b** in 80% yield in both reactions. Tebbe olefination of esters **143a** and **143b** yielded enol ethers **144a** and **144b** in 80% and 86% yield respectively. Activation of **144a** and **144b** with methyl triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine resulted in the oxocarbenium ion intermediates **145a** and **145b**, which after carbocylisation and elimination of the resulting carbocation **146a** and **146b**, resulted in formation of enol ethers **147a** and **147b** in 64% and 75% yield respectively. Hydroboration of **147a** and **147b** afforded protected 5'a-carbadisaccharides **148a** and **148b** in 72% and 78% yields, respectively. Two step deprotection of **148a** and **148b** gave the target 5'a-carbadisaccharides **149a** and **149b** in 72% overall yield in both cases (Scheme 24).[44]

1.4 Diels-Alder cycloaddition approach

In 1993, the Stoodley, Larsen *et al* jointly reported an attractive synthetic methodology for making pseudodisaccharides. Their methodology is based on the Diels-Alder cycloadditon between a sugar diene, such as **151** and maleic anhydride **150** to give cycloadducts **152** and **153**. The subsequent functionalisation and manipulation of the cycloadducts (**152** and **153**) gave the target pseudodisaccharide **154** (Scheme 25).[47]

Their strategy relies on the ability to chemically distinguish the two carboxylic acid groups of the anhydride functionality in cycloadducts **152** and **153**. This can be achieved either by reducing one group and decarboxylating the other, or reducing one group followed by decarboxylative amination at the other. This allows the synthesis of pseudodisaccharides with different possible connectivities of the carbasugar moiety to the true 'sugar' unit (Scheme 26).[47,48,49,50]

For example the Diels-Alder cycloaddition between **150** and **151** in benzene gave **152** and **153** as a 6:1 mixture in favour of the *endo*-cycloadduct (Scheme 27).[47]

Acidic hydrolysis of the silyl enol ether of the *endo*-cycloadduct **152** gave the corresponding ketone **155**. Treatment of ketone **155** with sodium cyanoborohydride in acetic acid was reported to afford lactonic acid **156** in 70% yield after recrystallisation, as the major product (see also Scheme 30). Conversion of compound **156** to the corresponding acid chloride using oxalyl chloride followed by treatment with sodium azide in anhydrous THF afforded acyl azide **157**. Curtius rearrangement of acyl azide **157** proceeded with retention of stereochemistry at C-4 and afforded isocyanate **158** in 98% yield. A one pot isocyanate and lactone reduction, using excess lithium aluminium hydride, followed by acylation gave a low yield of the desired product. Instead, however, selective hydrolysis of isocyanate **158** to the corresponding amine **159**, by

treating with triethylamine in aqueous THF gave a modest but workable yield of **159** (35%). Treatment of **159** with lithium aluminium followed by acetylation gave the (1→3) acetal-linked pseudodisaccharide **160** in 82% yield. Full deprotection of **160** gave the target pseudodisaccharide **161** in 73% yield (Scheme 28).[47]

For the synthesis of fully substituted (1→4) pseudodisaccharide s, Stoodley and Larsen first utilised allylic oxidation reaction to install a protected hydroxyl functional group at C-6 of the cycloadduct **152** (Scheme 29).[48] Thus, treatment of **152** with lead (IV) acetate in boiling DCM introduced an acetoxy group at C-6 to give **162**. Then, treating **162** with dimethyldioxirane followed by acetic anhydride and catalytic amount of perchloric acid installed an acetoxy group at C-4. Treatment of ketone **163** with sodium cyanoborohydride gave exclusively δ-lactonic acid **164**. In contrast to the formation of γ-lactone that was observed for **156**, intermediate **164** affords δ-lactone **165**. Decarboxylation at the C-6 of **166** gave the desired product **167** and a sulfide by-product **168** in 60% and 5% yields, respectively. Subsequent reduction of **167** with lithium aluminium hydride followed by acetylation afforded the protected (1→4) acetal-linked pseudodisaccharide **169** (Scheme 29). [48]

The substituent position on the cyclohexane ring of the cycloadduct influences the regioselectivity of the ring opening of the anhydride, when it was treated with sodium cyanoborohydride. For instance, when there is no substituent at C-6 and C-4, or there is a substituent at C-4 but not on C-6 of the anhydride, treatment with sodium cyanoborohydride results in a mixture of γ-and δ-lactone acids.[49] For example, treatment of **155** in glacial acetic acid with sodium cyanoborohydride resulted in a 83:17 mixture of γ-and δ-lactone acids **156** and **170** respectively and treatment of **171** in

glacial acetic acid with sodium cyanoborohydride resulted in a 75:25 mixture of γ- and δ-lactone acids **172** and **173** respectively (Scheme 30).[49] In contrast, when there is a substituent at both C-4 and C-6 as in **163**, treatment with sodium cyanoborohydride gives exclusively δ-lactonic acid **165** as shown in Scheme 29.[47]

Using the same methodologies developed previously and described above, the Stoodley and Larsen reported in 2001 the synthesis of (1→3) acetal -linked pseudodisaccharides **174** and **176**, starting from **171** and **175** respectively (Scheme 31).[49]

Another route to the synthesis of (1→3) acetal-linked pseudodisaccharides is the decarboxylation of the carboxyl group (b) of the anhydride functionality of **155**, and reducing that of (a) to the corresponding alcohol (see Scheme 28). The former can be achieved using the Barton decarboxylation reaction.[50] To this end, δ-lactonic acid **156** was treated with acid chloride followed by *N*-hydroxypyridinethione to yield light sensitive thiohydroxamic ester **177** in 92% yield. Treatment of **177** in the presence of *tert*-butyl thiol (a hydrogen donor) with light from a tungsten filament lamp afforded the decarboxylated product **178** in nearly quantitative yield (99%). Lithium aluminium hydride reduction of **178** followed by peracetylation gave the desired a (1→3) pseudodisaccharide **179** (Scheme 32).[50] It is worth noting that **179** can also be considered as (1→1) acetal linked pseudodisaccharide depending on the numbering of

the carbasugar unit of the pseudodisaccharide. A similar approach was adapted to prepare compound **183**, starting from **180** (Scheme 32).[50]

For the synthesis of (1→3) acetal -linked pseudodisaccharides with a hydroxyl substituent at C-4 (pseudodisaccharide numbering), the group have used the decarboxylation method developed by Barton where the radical produced from the decomposition of thiohydroxamic ester is trapped using oxygen.[51] Treatment of an oxygen-saturated solution of **177** with *tert*-butyl thiol and dimethylsulfide afforded alcohol **184** in 69% yield. Lithium aluminium hydride reduction of **184** followed by acetyl protection gave the desired (1→3) pseudodisaccharide **185**. A similar approach was adapted to synthesise compound **188**, starting from **186** (Scheme 33).[50]

Synthesising pseudodisaccharides through a Diels-Alder cycloaddition of a sugar diene and maleic anhydride **150** has its drawbacks; the resulting cycloadducts **152** and **153** have one extra carbon compared to the target pseudodisaccharide and elimination of the extra carbon makes the synthesis longer. To overcome this problem, Stoodley group reported the use of methyl (*E*)-3-nitroacrylate **192** in the synthesis of pseudodisaccharides.[52]

Dienophile **192** was prepared from methyl acrylate **189** in a three step synthesis (Scheme 34). Dienophile **192** underwent a smooth Diels-Alder cycloaddition with diene **151** to afford a 43:18:30:9 mixture of cycloadducts **193**, **194**, **195** and **196** (by

NMR analysis of the crude product) (Scheme 35).[52] Acidic hydrolysis of these cycloadducts gave a 42:18:28:12 mixture of ketones **197**, **198**, **199** and **200**.[52]

The major ketone **197** was isolated and converted into the corresponding (1→3) acetal-linked pseudodisaccharide **203**. First, ketone **197** was treated with sodium borohydride in methanol at -78 ^{o}C to afford the alcohol **201** in 74% yield. Reduction of the nitro group with aluminium amalgam in aqueous methanol followed by acetylation gave the hydroxylamine and amine derivatives **202** and **203** in 37% and 26% yield respectively. Treatment of the products (**202** and **203**) with lithium aluminium hydride in THF and

acetic anhydride in pyridine gave the target (1→3) acetal-linked pseudodisaccharide **204** (Scheme 36).[52]

1.5 Conclusion

Research into pseudodisaccharides is an evolving and expanding area. As we have seen throughout this chapter, there are now a number of different methods for the synthesis of pseudodisaccharides. Nevertheless, there is still a need for the development of other general and efficient methods, which would give easy access to different connectivity of a carbasugar to the "true" sugar component without relying excessively on the use of protecting groups.

Chapter Two: A New Synthesis of Pseudodisaccharides

2.1 Introduction & objectives

In this chapter, we will demonstrate the development of a general preparation of pseudodisaccharides through the Diels-Alder cycloaddition between vinyl sugars and appropriately substituted pyran-2-ones, to give the corresponding cycloadducts, followed by subsequent manipulation of these cycloadducts (Scheme 37).

Compared to the other methods (discussed in chapter 1), we envisaged that cycloaddition of a suitably substituted pyran-2-one to a vinylated sugar would be an advantageous mean of preparing pseudodisaccharides. First, the Diels-Alder cycloadducts of pyran-2-ones are functionally rich and are ideal springboards for the synthesis of a diverse range of carbasugar components of a pseudodisaccharide. In addition, cycloadditions can benefit from the induction of chirality by the enantiopure vinyl sugar dienophile, to afford the required pseudodisaccharides as single enantiomers. Compared with the coupling method, for example, this approach circumvents the need to prepare the carbasugar unit in an enantiomerically pure form, thus saving on the number of chiral interventions in the synthetic route.

In this chapter, we will first introduce the general topic of Diels-Alder cycloaddition reactions. We will then report the preparation of vinyl sugars and pyran-2-ones. This will be followed by the cycloadditions of vinyl sugars and pyran-2-ones and the investigations into the regio- and stereoselectivity of the reactions. Finally we will discuss the manipulation of the cycloadducts to afford the target pseudodisaccharides.

2.2 Diels-Alder cycloaddition

2.2.1 General overview

The Diels-Alder (DA) cycloaddition reaction is one of the most important reactions in organic chemistry. In 1906, Albrecht reported the thermal reaction between cyclopentadiene and *p*-benzoquinone. Although it was not realised at the time, this was the first reported example of a [4π+2π] cycloaddition.[53] Later, in 1928, Otto Diels and Kurt Alder disclosed the cycloaddition reactions of conjugated dienes with a number of alkenes.[54] Among the reactions they reported, was the cycloaddition reaction between cyclopentadiene **205** and maleic anhydride **150** to give cycloadduct **206** (Scheme 38).[54] Since then, the Diels-Alder reaction has found countless applications in organic chemistry. In recognition of their pioneering work, Diels and Alder were awarded the Nobel Prize for chemistry in 1950.

Cycloaddition reactions occur between a diene and a dienophile to form a cycloadduct. During the reaction, two new σ-bonds and a new π-bond are formed at the expense of the three π-bonds. The Diels-Alder cycloaddition is formally a concerted pericyclic reaction. It is expected that in a DA cycloaddition, the substituent on the diene or dienophile would not influence the reaction. However, in practice this is not the case. In particular, both the regio- and stereoselectivity of the reaction can be improved by matching the electronic demand of the substituents on the diene and dienophile. This matching of substituents can be done in two ways; therefore the DA cycloadditions can be divided into two types. “Normal” electron demand DA cycloadditions are those in which the dienophile bears an electron deficient group and the diene bears an electron rich group. In “inverse” electron demand DA cycloadditions, the dienophile bears an electron rich group and the diene bears an electron deficient group. It should be noted that the term “normal” does not necessarily imply prevalence or typicality of the reaction. It is simply a consequence of those types of DA reaction being historically the first to be observed. Indeed, from a frontier molecular orbital (FMO) point of view, both reactions carry the same validity.

The outcome of a DA cycloaddition can be predicted through FMO theory. FMO theory states that the reactions are encouraged when there is a favourable interaction between the highest occupied molecular orbital (HOMO) of one component and the lowest unoccupied molecular orbital (LUMO) of the other based on their energy separation.[55]

Energy separations in a normal electron demand cycloaddition.

Energy separations in an inverse electron demand

In normal electron demand cycloadditions, which involve an electron rich diene and an electron deficient dienophile, the HOMO of the diene interacts with the LUMO of the dienophile because of its smaller energy gap compared with the HOMO (dienophile)-LUMO (diene). In inverse electron demand cycloadditions the LUMO of the diene interacts with the HOMO of the dienophile. In general, an electron donating groups (EDG) increase the relative energy of the HOMO by promoting electron density, and an electron withdrawing groups (EWG) reduce the relative energy of the LUMO by removing the electron density. Thus, substituting one of the components of the DA cycloaddition with an electron withdrawing group, and the other with an electron donating group (matching the electronic demands) would significantly facilitate the reaction (Scheme 39).

2.2.2 Regioselectivity of Diels-Alder reactions

The cycloaddition of unsymmetrical dienes with unsymmetrical dienophiles should result in two regioisomeric cycloadducts. In practice we normally observe the

predominant formation of one. This is explained by the assumption that the DA cycloadditions are *asynchronous*, which means that in the process leading to the formation of the transition state one bond forms faster than the other. The *asynchronicity* of the bond formation is predicted through polarity of the atoms involved in this "pre-bonding" arrangement. For example, the DA cycloaddition between methyl acrylate **189** and 1-methoxybuta-1,3-diene **207** results in the 1,2-substituted cycloadduct **208** as the major product. This can be explained by the way the molecules arrange themselves prior to the formation of the transition state. The major product is derived from the organisation of the molecules in such a way that the interactions of partial charges are complementary (Scheme 40).

The *asynchronicity* in the DA cycloadditions can be shown computationally. For example, our group has previously shown that the transition state leading to the formation of the 5-*endo*-cycloadduct from the reaction between 5-iodopyrone and methyl acrylate is very *asynchronous* (Figure 11).[56]

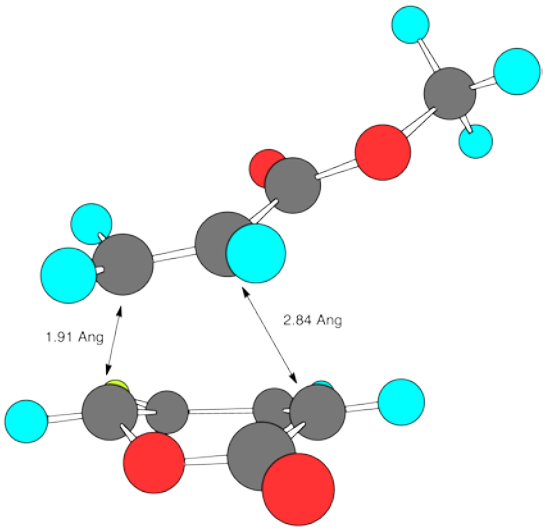


Figure 11

2.2.3 Stereoselectivity of Diels-Alder reaction

DA cycloadditions are also stereoselective. In many DA reactions the *endo*-product is the major product. This observation is a corollary to Alder's *endo* addition rule, which states that the two components arrange themselves in parallel planes in the transition state, and the most stable transition state arises from the orientation with the maximum accumulation of double bonds. The formation of the two new σ bonds is a result of the primary interactions between the orbitals of the diene and dienophile. However, there is an additional interaction between the π-orbitals of the alkene substituent that lies below the diene. These 'secondary orbital interactions' energetically contribute to the stabilisation of the transition state with the *endo*-orientation (Scheme 41).

Under kinetic conditions, the *endo*-isomer is formed at a fast rate, as its activation energy is lowered due to favourable secondary orbital interactions. However, under thermodynamic conditions (for example prolonged reaction times, higher temperature), the *exo*-isomer may be formed. Under these conditions the cycloaddition can become reversible, which means that the thermodynamically most stable product will dominate. Since in the majority of DA cycloadditions (and particularly in the cycloadditions of pyran-2-ones), the *exo*-isomer is sterically less congested than the *endo*-isomer, it would be the most stable and hence the favoured product under thermodynamic conditions. Therefore, a degree of care must be taken in the analysis of the outcome of DA cycloadditions, particularly those with a longer reaction time. A good example is the

cycloaddition between 1-ethyl-3-methylpyridone **210** and 1-phenyl-1*H*-pyrrole-2,5-dione **211**, where *endo*-cycloadduct **212** is formed at a lower temperature rather than the *exo*-cycloadduct **213**. Furthermore, the *endo*-cycloadduct is converted into the *exo*-cycloadduct when heated at a higher temperature (Scheme 42).[57]

2.3 Preparation of appropriate dienes and dienophiles

As described in the introduction (section 2.1), we want to develop a general synthetic methodology towards pseudodisaccharides, through DA cycloadditions between vinyl sugars and pyran-2-ones as the key step (Scheme 43). Obviously, the alkene dienophile component in this reaction is electron rich as the methodology dictates that it would be as it is substituted with an oxygen atom. In order to maximise the efficiency and selectivity of the cycloaddition, the diene component must be electron deficient.

The electronic demand of unsubstituted pyran-2-one (**214**, $R^1 = R^2 = H$) has not been experimentally investigated in detail, because it is highly prone to polymerisation. However, 3- and 5- halo substituted pyran-2-ones (**214**, R^1 or R^2 = hal) are known to be ambident dienes, reacting with electron rich dienophiles including vinyl ethers, as well as electron deficient ones.[56, 58] Since removal of the halogen in a later step in the synthesis is possible, 3- and 5- halo substituted pyran-2-ones are considered as the synthetic equivalents of pyran-2-ones in both normal and inverse electron demand cycloadditions. Therefore, one approach could involve the use of 3- and 5- halo substituted pyran-2-ones as the diene component.

However, 3- and 5- halo substituted pyran-2-ones are relatively unreactive in DA cycloadditions. Therefore, we felt it was appropriate to also investigate the DA cycloadditions of pyran-2-ones substituted with highly electron deficient carbomethoxy and carboethoxy substituents in the 3- and 5- positions. Although this approach is more likely to afford efficient and selective cycloadditions, the cycloadducts would contain one extra carbon atom than required for carbasugar synthesis. Therefore a degradation reaction must be carried out at some stage in the chemical manipulation steps that follows the cycloaddition.

2.3.1 Preparation of Dienophiles (vinyl sugars)

To exemplify the methodology, we decided to use three vinyl sugars. Sugar molecules were appropriately protected and then vinylated at three different positions: C-6 hydroxyl, C-3 hydroxyl and the anomeric hydroxyl.

2.3.1.1 Preparation of 3-vinyl glucofuranose

Anhydrous D-glucose **215** was stirred in excess acetone and concentrated sulphuric acid to afford glucose diacetonide **216** (Scheme 44).[59] Under acidic conditions, the 6-membered pyranose ring is in equilibrium with the open chain and 5-membered ring furanose forms. The furanose form reacts with acetone to give two 5-membered ring cyclic acetonides. The remaining acetonide protection is installed by reaction of the C-5 and C-6 hyrdroxyl groups of glucose.

The C-3 hydroxyl of **216** was vinylated using a literature procedure.[60] There are several methods to vinylate monosaccharides, including using a selenium reagent,[61] the Hofmann elimination of *N*-2-trimethylammonium-ethyl-*O*-glycoside,[62] photochemically induced reactions[63] and a mercury acetate mediated reaction of isobutyl vinyl ether with sugar molecules.[64] Other routes to vinyl sugars employ TMS-triflate promoted elimination reactions of mixed acetal glycosides[65] and palladium(II) acetate catalysed vinylation of monosaccharides.[60]

We have used the palladium(II) acetate catalysed vinylation method developed by Schlaf *et al.*[60] 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **216** was dissolved in excess butyl vinyl ether (BVE) **217** in the presence of 4,7-diphenyl-1,10-phenanthroline ligand and palladium(II) acetate in a sealed flask and was stirred at 75 ^{o}C for 7 days.

The crude reaction mixture was passed through activated charcoal to remove solid materials. After removal of the excess BVE, the crude product was purified on deactivated silica gel by pre-treating it with petrol/ethyl acetate/triethyl amine (92:6:12) as the eluent system, to afford 3-vinyl α-D-1,2:5,6-di-*O*-isopropylidine glucofuranose **218** in 88% yield (Scheme 44).

A mechanism for the reaction is proposed in Scheme 45. In the reaction, the 4,7-diphenyl-1,10-phenanthroline ligand co-ordinates to the palladium(II) acetate and increases its coupling efficiency. The catalytic transfer vinylation is thought to proceed in the oxypalladation-deoxypalladation pathway in which both intra- and intermolecular attack of the exchanging alcohol leads to the formation of the palladium alkyl complex intermediate. It can then either generate the product through the rotation of carbon-carbon double bond followed by deoxypalladation, or go back to the starting alcohol (Scheme 45).[60]

2.3.1.2 Preparation of 6-vinyloxy-β-D-glucose-1,2,3,4-tetraacetate

We utilised the method developed by Evans *et al*[66] to prepare a glucose molecule protected at all the hydroxyl groups except the C-6 hyroxyl. First a mixture of anhydrous glucose **215** and trityl chloride in pyridine was refluxed to give the trityl protected C-6 hyroxyl. The reaction proceeds with the formation of the trityl carbocation, which is stabilised through delocalisation of the positive charge, followed by nucleophilic attack by the oxygen lone pair. The selectivity of the reaction arises from the fact that the trityl carbocation exists in a sterically congested propeller shape to avoid disfavourable steric interactions (Figure 12). Therefore the trityl cation reacts

with the least sterically demanding hydroxyl group which in this case is the primary C-6 hyroxyl.

Figure 12

The remaining hydroxyl functions were acetylated in the same reaction pot by treatment with acetic anhydride in the presence of base. The two diastereomers (α and β anomers) were separated by triturating the crude reaction mixture with ether. The α-isomer is soluble in ether whereas the β-isomer is not. The ether-insoluble residue was then recrystallised from hot ethanol, to afford 42% yield of the β-isomer **219** (Scheme 46).

Finally, the C-6 hydroxyl was deprotected by treating **219** with a solution of hydrobromic acid in acetic acid to afford **220** in 53% yield (Scheme 48). Once the C-6 hydroxyl was free, it was vinylated using the method described above to afford after recrystallisation the 6-vinyloxy-β-D-glucose-1,2,3,4-tetraacetate **221** in (45%) yield after recrystallisation from petrol (Scheme 47).[60]

2.3.1.3 Preparation of anomerically pure vinyl sugar

A solution of D-mannose **222** in pyridine was treated with benzoyl chloride and catalytic amount of DMAP and stirred overnight at room temperature to afford, after recrystallisation, pentabenzoate **223** as a single anomer in 72% yield.[67] Compound **223** was selectively deprotected without loss of anomeric purity using ethanolamine to afford tetrabenzoate **224** in 50% yield. The anomeric purity of **224** was established by proton and carbon-13 NMR. The anomeric hydroxyl was subsequently vinylated using the methodology described above to afford the corresponding vinyl sugar **225** (Scheme 48).[60]

2.3.2 Synthesis of pyran-2-one

Four 2(*H*)-pyran-2-one derivatives were chosen as the diene component for the Diels-Alder cycloaddition with vinyl sugars. One of those chosen was the commercially available 3-carbomethoxy-2(*H*)-pyran-2-one **226**. The other three dienes were ethyl coumalate **227**, 3-bromo-2(*H*)-pyran-one **228** and 5-bromo-2(*H*)-pyran-one **229** (Figure 13). All the three dienes were prepared from literature procedures.[68,69]

Figure 13

2.3.2.1 Preparation of ethyl coumalate

A solution of coumalic acid **230** in DCM was treated with 4-dimethylamine pyridine (DMAP), dicyclohexylcarbodiimide (DCC) and ethanol, and was stirred for 17 h.[69] After work-up and chromatographic purification the desired ethyl coumalate **227** was obtained in 75% yield (Scheme 49).

2.3.2.2 Preparation of bromo-2(*H*)-pyran-2-one

3-Bromo-2(*H*)-pyran-2-one **228** and 5-bromo-2(*H*)-pyran-2-one **229** were obtained from the commercially available 5,6-2dihydro-2(*H*)-pyran-2-one **231** in the following sequence of reactions (Scheme 50).[68] First, 5,6-dihydro-2(*H*)-pyran-2-one **231** in DCM was treated with bromine and stirred at room temperature for 8 hours. Then the reaction mixture was treated with Et_3N and stirred at room temperature for a further 15 h to afford **232** in 91% yield. Then **232** was subjected to radical bromination by treating it with NBS **234** and benzoyl peroxide **235** and heating the reaction mixture at 100 ^oC for 4 hours to afford 3,5-dibromo-5,6-dihydro-2(*H*)-pyran-2-one **233** in 67% yield.

The initiation step involves the breakdown of benzoyl peroxide **235** to form radical **236** on heating. Radical **236** reacts with **231** to generate radical **237** and benzoic acid **238**. The propagation step involves the reaction of *N*-bromosuccinimide **234** with **237** to form 3,5-dibromo-5,6-dihydro-2(*H*)-pyran-2-one **233** and succinimide radical **239** (Scheme 51). The termination step involves all radicals reacting with each other. Treatment of 3,5-dibromo-5,6-dihydro-2(*H*)-pyran-2-one **233** with Et_3N results in the formation of 3-bromo-2(*H*)-pyran-2-one **228** as a major product (33%) and 5-bromo-2(*H*)-pyran-2-one **229** as a minor one (7%).

Initiation:

Propagation:

5-Bromo-2(*H*)-pyran-2-one **229** can be prepared more efficiently in one step directly from 5,6-dihyro-2(*H*)-pyran-2-one **231** (Scheme 52). The yield of the reaction was low (22%) due to the formation of 3,5-bromo-2(*H*)-pyran-2-one **241** as a major by-product (Scheme 53).[70]

.

2.4 Diels-Alder Cycloaddition between vinyl sugars and pyran-2-ones

2.4.1 Reaction conditions and selectivities

Once the vinyl sugars and pyran-2-ones were in hand, we investigated their Diels-Alder cycloadditions. Cycloadditions of vinyl sugars with pyran-2-ones were carried out in a sealed tube in dichloromethane over a temperature range of 45-100 ºC for 5-7 days. Typically, two equivalents of the dienophile were used, although the quantity of the dienophile was increased for very slow reactions (Scheme 54). Outcome of the reactions were determined by analysis of the ^{1}H-NMR spectra of the crude reaction mixtures (see section 2.4.2). The results of the cycloadditions between vinyl sugars and pyran-2-ones are summarised in Table 3.

Since the dienophiles (vinyl sugars) are single enantiomers, the cycloadditions can afford 4 possible products. There will be one pair of 5-*endo* cycloadducts depending on which face of the dienophile is presented to the diene. There could also be a pair of *exo*-cycloadducts. The four cycloadducts are obtained as single enantiomers, however the

relationship between them is diastereomeric. For the purposes of this thesis, in order to distinguish the diastereofacial stereoisomers from *endo* and *exo* isomers, the cycloadducts are labelled *endo*-1 and *endo*-2, and *exo*-1 and *exo*-2. Of course, depending on the stereoselectivity of the reaction, not all four possible cycloadducts may be actually formed.

Table 3

a. There were traces of *exo* product in the reaction mixture but it was not possible to measure its abundance.

For example, the cycloaddition of 3-vinyl glucofuranose **218** with 3-carbomethoxy-2(*H*)-pyran-2-one **226** was carried out in a sealed tube in dichloromethane at 65 ºC over 7 days using 2 equivalents of the dienophile (Scheme 55). Analysis of the crude reaction mixture by NMR revealed it to contain a mixture of three cycloadducts in the

ratio of 3.4 **246a** : 2.0 **246b** : 0.1 **246c/d** as well as a small quantity of unreacted pyrone **226**. Following chromatographic separation on silica gel, the *endo*-configuration for cycloadducts **246a** and **246b**, were established by analysis of the coupling constants in the 600 MHz ^{1}H NMR (see later section 2.4.2).[71,72] Similarly, the *exo*-configuration for the cycloadducts **246c/d,** a mixture of two diastereomers which could not be separated by chromatography, was established.

The absolute configuration of the minor *endo*-cycloadduct **246b** was established unequivocally by X-ray crystallography as (1*R*, 5*S*) (Figure 14). The major *endo*-cycloadduct **246a** was an oil; however, as can be seen later, the absolute configuration

of this isomer was established retrospectively as (1*S*, 5*R*) based on X-ray crystallography on a derivative (see Figure 19).

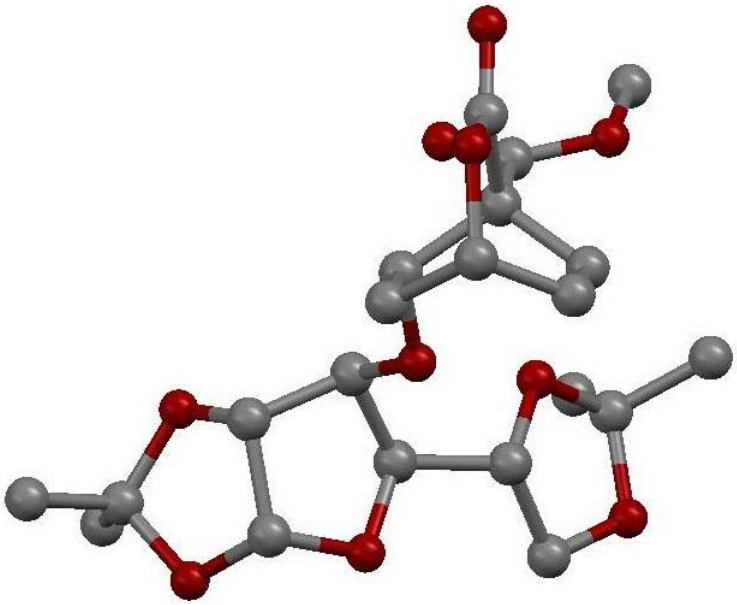


Figure 14: X-ray structure of cycloadduct **246b**

To rationalize this modest diastereofacial selectivity, we can assume that the approach of the pyrone diene **226** to the vinyl ether **218** is controlled by the steric bulk of the sugar's 5',6'-*O*-isopropylidene substituent, rather than the 1',2'-*O*-isopropylidene substituent. This is presumably because the former can exert more steric hindrance due to possible rotation about C4'-C5' bond (Figure 15).

Figure 15

On the other hand Diels-Alder cycloaddition between 3-vinyl glucofuranose **218** and ethyl coumalate **227** afforded 4 cycloadducts (2 *endo* and 2 *exo*). Although we could separate the *endo*- and the *exo*-isomers from one another, it was not possible to separate the diastereofacial diastereomeric pairs, affording a mixture of *endo*-1 & 2 and a mixture of *exo*-1 & 2 (Scheme 56).

The cycloaddition of 6-vinyloxy-β-D-glucose-1,2,3,4-tetraacetate **221** with all four pyran-2-ones is stereoselective and only affords the *endo*-cycloadducts (Table 3). However, the cycloaddition lacks diastereofacial selectivity and affords a mixture of 1:1 *endo*-1/*endo*-2 which could not be separated by chromatographic means. This lack of diastereofacial selectivity may be explained by the observation that the reaction centres are too far away from the chiral centres of the sugar component.

On the other hand the cycloaddition between *O*-benzoyl-α-D-mannopyranose **225** and 3-carbomethoxy-2(*H*)-pyran-2-one **226** does afford both *endo*- and *exo*-cycloadduct.

Generally speaking, 5-bromo-2(*H)*-pyran-2-ones **228** and 3-bromo-2(*H*)-pyran-2-ones **229** were not as reactive towards vinyl sugars as 3-carbomethoxy-2(*H*)-pyran-2-one **226** and ethyl coumalate **227** and 10 to 20 equivalents of the dienophile was needed for the reactions to occur. However, no cycloaddition reaction was observed between **225** and 3-bromo or 5-bromo-2(*H*)-pyran-2-ones even using 20 equivalents of the dienophile, higher temperature (>100 ^{o}C) and longer reaction time (i.e >10 days).

2.4.2 Determination of the cycloadduct structure

As was discussed in the previous section, ratios of the isomers were determined from the analysis of the ^{1}H-NMR of the crude reaction mixtures. In this section, we provide the rationale for these assignments.

Through previous studies of the Diels-Alder cycloaddition of pyran-2-ones, our group and others[71,72,73] have developed a set of empirical rules for the determination of the *endo*- and *exo*-configuration of the cycloadducts. These rules involve the comparison of both the chemical shifts and the coupling constants of the protons in the bicyclic lactone structure.

The rules are most reliable if both the *endo*- and *exo*-isomers are at hand so that a direct comparison of the chemical shifts and the coupling constants can be made. However, even if only one of the cycloadducts is present, the empirical rules can fairly reliably predict its *endo*- or *exo*-configuration. Indeed, through the extensive experience in our group of the pyran-2-one cycloadditions, there have only been a handful of cases, where it has not been possible to assign the configuration of the cycloadducts.

Figure 16

The empirical rules are derived from two observations about the chemical shifts and coupling constant in the bicyclic lactone cycloadducts: The first is that the protons in the *endo*-position of the saturated bridge in 2-oxabicyclo[2.2.2]oct-7-en-3-one (protons H-5_{endo} and H-6_{endo}) are influenced by the magnetic anisotropy of the C7-C8 double bond and therefore resonate at lower frequency than would be expected from their connectivity to various atoms and groups. In contrast, protons H-5_{exo} and H-6_{exo} are not influenced by the magnetic anisotropy of the C7-C8 double bond and therefore, resonate at the chemical shift expected from their connectivity to various atoms or groups.

The second observation is that the coupling constants of the protons at the bridgeheads to the protons at the *endo*-position are usually smaller than those of the protons in the *exo*-position (Figure 16). This is presumably a consequence of the shorted C-O bond in the bridging ester and the boat conformation of the ring. This results in an increase in the dihedral angle between CH-5_{endo} and CH-6_{endo} with their respective bridgehead CHs whilst causing a reduction in the dihedral angle between CH-5_{exo} and CH-6_{exo} with their respective bridgehead CHs (Figure 17). Based on these two observations, we can use the following protocol to identify the *endo*- and *exo*-isomers.

Figure 17: definition of dihedral angle α

There are two possible scenarios. The first one is when there is no substituent at C-4 of the cycloadduct (e.g. a cycloadduct from the reaction of a 5-substituted pyran-2-one) and the second one is when there is a substituent at C-4 (e.g. a cycloadduct from the reaction of a 3-substituted pyran-2-one) (Figure 18). In the first case, we look at the coupling between H-4 and H-5. If $J_{4,5}$ is larger than 2.0-2.5 Hz, then H-5 is in the *exo*-face and our cycloadduct is *endo*. If $J_{4,5}$ is smaller than 2.0-2.5 Hz, then H-5 is in the *endo*-face and our cycloadduct is *exo*. If both cycloadducts were isolated, then their configuration can also be confirmed by the analysis of their chemical shifts. H-5 in the *exo* cycloadduct has a lower chemical shift than the H-5 in the *endo*-cycloadduct.

Figure 18

On the other hand when there is a substituent at C-4, we use a more involved analysis. We can first identify the signals for H-1 and the two H-6s. The signal due to H-1 appears as a multiplet at about 5 to 5.5 ppm. For most cycloadducts there are no other major signals in this region and H-1 signal can be easily identified. The signals due to the two H-6s are also easily identifiable. They are the only two signals in the 1.5 to 3 ppm region that have a large *gem* coupling of about 12-14 Hz.

We then use the coupling between H-1 and each of the H-6's to distinguish H-6_{exo} from H-6_{endo}. If one of the H-6s has a $J_{1,6}$ coupling which is larger than 2.0-2.5 Hz, then that proton is in fact H-6_{exo}. If one of the H-6s has a $J_{1,6}$ which is smaller than 2.0-2.5 Hz, then that proton is in fact H-6_{endo}. In addition, H-6_{endo} is also expected to have a lower chemical shift than H-6_{exo}.

Once we have identified which signal is H-6_{endo} and which is H-6_{exo}, we use the size of their coupling to H-5 to determine H-5s configuration. If H-5 couples to H-6_{endo} by a value about 8-10 Hz and to H-6_{exo} by a value about 4-7 Hz, then H-5 and H-6_{endo} are *syn* to each other and H-5 is also in the *endo*-face, and we have the *exo*-cycloadduct. However, if H-5 couples to H-6_{exo} by a value about 8-10 Hz and to H-6_{endo} by a value about 4-7 Hz, then H-5 and H6_{exo} are *syn* to each other and H-5 is also in the *exo*-face and we have the *endo*-cycloadduct.

2.5 Chemical manipulation of the cycloadducts

Once the cycloadducts were in hand, their conversion to the corresponding pseudodisaccharides was investigated. In order to demonstrate the applicability of the methodology, we decided to carry out chemical manipulation of cycloadduct **246a** to gain access to the (3→4) linked pseudodisaccharide **255** (Scheme 57).

However, cycloadducts **246a** and **246b** contain 8 carbon atoms instead of 7 carbon atoms in their corresponding carbasugar unit of the pseudodisaccharide; therefore it was necessary to carry out a degradation reaction to eliminate the extra carbon atom. This could be achieved by deallylation followed by decarboxylation of the ring opened cycloadduct using palladium (II) acetate.[74] Therefore, we first set out to demonstrate the applicability of the deallylation/decarboxylation step in these systems.

Cycloadduct **246a** was treated with lithium allyl oxide, prepared from freshly distilled allyl alcohol and butyl lithium, resulting in trans-esterification to afford diester **256** in 81% yield. The mechanism of this reaction involves nucleophilic attack of the alkoxide anion onto the ester carbonyl which results in the ring opening of the lactone molecule

and subsequent protonation of the resulting alkoxide ion after acidic workup (Scheme 58).

The one pot deallylation/decarboxylation of **256** was attempted by heating **256** at 100 ^{o}C with palladium(II) acetate, triphenylphosphine, formic acid and triethylamine in a sealed tube for 18 h to afford unsaturated ester **261** as a crystalline compound in 63% yield (Scheme 59).

In this reaction, palladium metal coordinates the allyl group in diester **256** causing deallylation of **256** to generate carboxylate **258** and palladium complex **257**. This is followed by decarboxylation of **258** to give **259**. Then acidic work up of **259** resulted the desired ester **260** and allyl formate **261** as well as regenerating the palladium (II) acetate catalyst **262** (Scheme 60).

At this stage, the structure of compound **260** was confirmed by X-ray crystallography (Figure 19). Since, compound **255** had originated from major *endo*-cycloadduct **246a** , it was now possible to confirm the absolute configuration of **246a** as (1*S*, 5*R*).

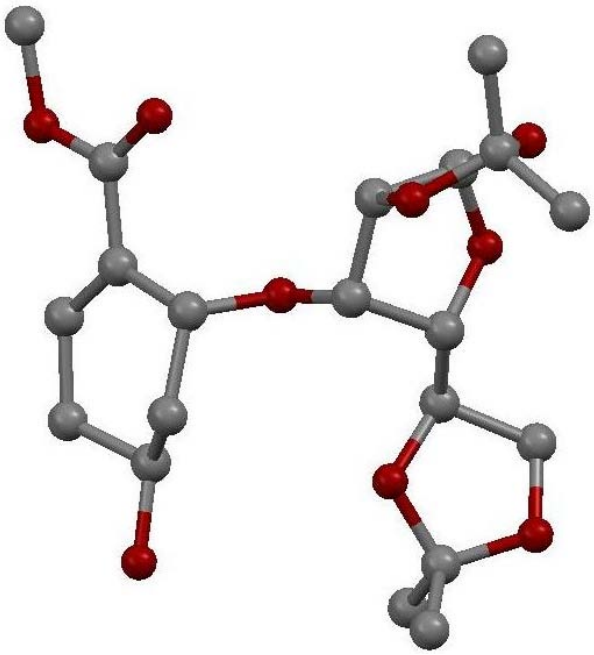


Figure 19: X-ray structure of compound **260**

Treatment of **260** in toluene with excess DIBAL-H at -78 ^{o}C then afforded allylic alcohol **263** in 88% yield (Scheme 61). Normally treatment of an ester group with DIBAL-H at low temperature furnishes the corresponding aldehyde. But, using excess DIBAL-H and a longer reaction time, as in this case, affords the corresponding alcohol.[74]

Finally, we undertook the stepwise deprotection of the two acetonides in **263**, leading to the formation of **264** initially and then to the fully deprotected pseudodisaccharide **255**.

Treatment of **263** with 90% acetic acid at room temperature for 18 h resulted in the removal of 5',6'-*O*-isopropylidene substituent of the sugar to give **264** in 90% isolated yield (Scheme 62). However, leaving the reaction for longer failed to remove the 1',2'-*O*-isopropylidene.

It is known that the 5',6'-*O*-isopropylidene substituent is easily removed than the 1',2'-*O*-isopropylidene substituent.[75] This is generally attributed to entropic factors since in the former there is rotation about C4'-C5' bond at the oxonium intermediate stage (Scheme 63).

The removal of 1',2'-*O*-isopropylidene substituent was achieved by treating **264** with 50% aqueous TFA at 0 ^{o}C for 30 minutes and then elevating the temperature to room temperature for another 30 min. This afforded **255** in 80% yield as a mixture of anomers (Scheme 64).

Using the same methodology, pseudodisaccharide **268** was prepared starting from the minor *endo*-cycloadduct **246b** (Scheme 65).

We next attempted to dihydroxylate **246a** at the C7-C8 double bond by treating **246a** in acetone/water with osmium tetraoxide and 4-methylmorpholine *N*-oxide and stirred for 24 h. The reaction was quenched by addition of reducing agent aqueous sodium bisulfite followed by extraction (Scheme 66). The crude product was obtained as a crystalline compound. ^{1}H and ^{13}C NMR on this material confirmed the disappearance of the signals due to the C7-C8 double bond and the likely introduction of new substituents at (now saturated) C7 and C8. However, mass spectrometry failed to give the expected molecular ion and was inconclusive as to whether hydroxyl functions were present in the molecule. We tried to protect the dihydroxyl groups with acetonide group and the starting material decomposed. We also attempted to ring open the cycloadduct

and the starting material also decomposed. Also the compound in hand was very unstable and decomposed if it was left to stand in solution at room temperature. Therefore, we could not unequivocally assign the structure **269** to this compound.

2.6 Cycloaddition-cycloreversion reactions of Pyran-2-one

We saw in the previous section that 2(*H*)-pyran-2-ones undergo cycloadditions with vinyl sugars to initially afford functionally rich, bridged bicyclic lactone cycloadducts. We also demonstrated that the cycloadducts are useful springboards for the synthesis of pseudodisaccharides.

During our investigation of the Diels-Alder cycloaddition of 2(*H*)-pyran-2-ones with a vinylated sugar, we noticed a significant role played by the temperature of the reaction. For example, when the 3-vinyl glucofuranose **218** and 3-carbomethoxy-2(*H*)-pyran-2-one **226** were heated at 65 °C in a sealed tube for 7 days, it afforded cycloducts **246a**, **246b** and **246c/d** as discussed earlier. However, upon increasing the temperature to 80 ^{o}C, significant quantities of a second product, as a mixture of diastereomers **270a** and

270b, was also obtained. At 100 °C, the reaction quickly and efficiently afforded compounds **270a** and **270b** (Scheme 67).

2(*H*)-Pyran-2-ones, such as **226**, enjoy aromatic resonance stabilisation (Figure 20).[76] This aromatic stabilisation, estimated to be about 35-40 kJ/mol, contributes to the activation energy for the cycloadditions of 2(*H*)-pyran-2-ones. If forcing conditions are used in the cycloaddition reactions, cycloadducts may undergo two possible further reactions. One is the reversion to the starting 2(*H*)-pyran-2-one. This pathway results in a regain of aromatic resonance stabilisation energy and has been generally accepted that it is the favoured fate of the pyran-2-one cycloadducts at higher temperature. If this was the case, the cycloaddition becomes reversible. As discussed in section (2.2.3), under

reversible conditions, thermodynamically more stable *exo*-cycloadducts predominate. Indeed, the formation of *exo*-cycloadducts in pyran-2-one cycloadditions can be attributed mainly to this pathway as was shown by Susherina (Scheme 42).[57] The other pathway is the loss of bridging carbon dioxide in a retro Diels-Alder reaction. This affords the corresponding cyclohexadienes which are considered very reactive and either aromatize to afford benzenes,[71,77,78] or undergo cycloaddition with any excess dienophile present in the reaction mixture[79] (Scheme 68). This is in fact also a common reaction pathway and has been a very useful method for the synthesis of benzenes, although the methodology has not found significant application in the synthesis of non-aromatic six-membered rings, presumably due to the difficulty in controlling the synthesis of highly reactive cyclohexadiene intermediate.

Figure 20

Therefore after careful review of the spectroscopic data of compounds **270a** and **270b**, it was much unexpected when they were revealed to be indeed cyclohexadienes obtained

from the loss of bridging carbon dioxide. The structure of compound **270a** was subsequently confirmed by X-ray crystallography (Figure 21). This represents the first ever example of a crystal structure of a cyclohexadiene from a retrocycloaddition origin. Compound **270a** appears to be exceptionally stable. It withstands temperatures of up to 120 °C, is air stable over a period of months and could be isolated after chromatography in multi-gram quantities.

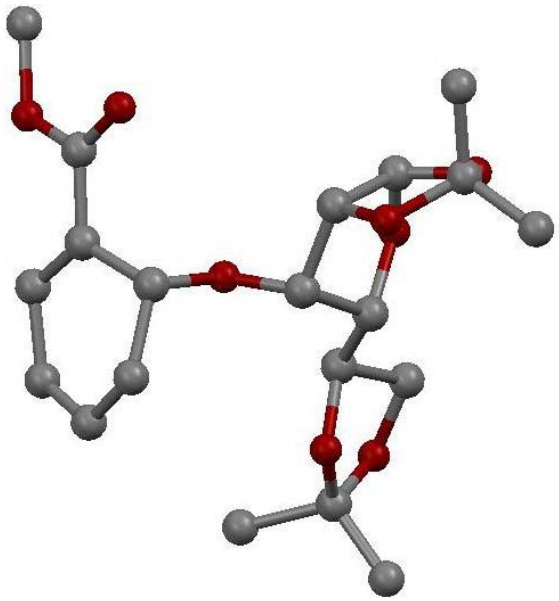


Figure 21: X-ray structure of compound **270a**

Interestingly, we also observed similar results in the cycloaddition between 6-vinyloxy-β-D-glucose-1,2,3,4-tetraacetate **221** and 3-carbomethoxy-2(*H*)-pyran-2-one **226**. Heating the reaction mixture at 45 ^{o}C for 6 days afforded the *endo*-cycloadducts **245a,b**. Increasing the temperature to 60 ^{o}C afforded a mixture of cycloadducts **250a,b** and cyclohexadienes **271a,b** in 1.2:1 ratio respectively (Scheme 69). The cyclohexadiene **271a,b** was isolated as a mixture diastereomers.

However, we observed that the cycloaddition between ethyl coumalate **227** and 3-vinyl glucofuranose **218** at elevated temperature afforded only a small amount of a decarboxylated products also as a mixture of two diastereomers **272a,b** (Scheme 70). In contrast, the loss of carbon dioxide was not observed in any of the cycloadditions of bromopyran-2-ones.

Loss of bridging carbon dioxide has been previously reported by a number of groups.[80] For example, Marko *et al*[81] have reported that heating cycloadduct **273** in toluene at 110 ^{o}C overnight causes the loss of bridging carbon dioxide to afford cyclohexadiene **274** (Scheme 71).

The unusual reaction pathway, favouring loss of bridging carbon dioxide rather than cycloreversion back to the starting pyran-2-one, prompted us to investigate the reaction further.

As discussed earlier, cyclohexadiene **270a** and **270a** were obtained as a diastereomeric mixture. Although cyclohexadiene **270b** is an oil, **270a** is crystalline and we were able to obtain a X-ray crystal structure of this compound to confirm its proposed structure and absolute configuration (Figure 19). The relative configuration of **270a** suggests that it is derived from the major cycloadduct **246a**. Nevertheless, we confirmed that **270a** is derived from the major cycloadduct **246a**, previously determined as (1*S*,5*R*), and compound **270b** is derived from the minor cycloadduct **246b,** previously determined as (1*R*, 5*S*), by heating each of the cycloadducts independently at 100 ^{o}C in sealed tubes for 7 days (Scheme 72). Reformation of the *exo*-product was not observed during conversion of either **246a** or **246b**.

However, during the loss of bridging carbon dioxide, the information regarding the stereochemistry of the C-1 of the cycloadduct is also lost. This means that **270a** can not only have originated from the *endo* **246a**, but also *exo* **246d**. Similarly, **270b** can not only have originated from the *endo* **246b**, but also *exo* **246c**. Since the ratio of

246a:**246b** at 65 ^{o}C is 1.7:1 whilst the ratio of **270a**: **270b** is 2.6:1 we cannot rule out that some conversion of the *endo*-cycloadducts to the *exo*-cycloadduct could have taken place prior to the loss of the bridging carbon dioxide.

One of the unexpected observations that we made was that cycloadducts of **218** with ethyl coumalate **227** appeared to be less prone to the loss of bridging carbon dioxide than those of **218** with 3-carbomethoxy-2(*H*)-pyran-2-one **226**. To ensure this difference was not driven by the steric bulk of the glucofuranose **218**, we also investigated the rates of decarboxylation of the cycloadducts from 3-carbomethoxy-2(*H*)-pyran-2-one **226** and ethyl coumalate **227** with butyl vinyl ether (BVE) **217** (Scheme 73). In the first instance, the reactions of **226** and **227** with **217** were carried out in a sealed tube at 100 ^{o}C and the progress of the reactions was monitored by NMR

at different time intervals. After 24 h, the reaction between **227** and BVE **217** was complete and only cycloadduct **275** was present in the reaction mixture. On the other hand, after 24 h in the reaction between **226** and BVE **217**, there was a mixture of cycloadduct **277** and decarboxylated product **278** in 1:1 ratio. After 48 h, there was still no decarboxylated product **276** in the reaction of **227** and BVE **217**. All the cycloadduct **277** has converted into the decarboxylated product **278** after 48 h in the reaction mixture of the cycloaddition between **226** and BVE **217**. We concluded that the decarboxylation reaction happens faster when the carboxyl substituent on the pyran-2-one is in the 3-position.

To gain a better understanding of the variation in the rate of the cycloaddition and those for the loss of carbon dioxide from the cycloadducts, we decided to experimentally and computationally investigate the reaction. As a preamble to these investigations, we isolated and characterised the *endo*-cycloadducts **275** and **277** in pure form after conducting the reaction at lower temperatures. We first investigated the rates of cycloadditions for both 3-carbomethoxy-2(*H*)-pyran-2-one **226** and ethyl coumalate **227**

with BVE **217** at three different temperatures. The temperatures were carefully chosen so that no decarboxylation could occur. For the cycloadditions of 3-carbomethoxy-2(*H*)-pyran-2-one **226** with BVE **217** and the cycloadditions of ethyl coumalate **227** with BVE **217** we chose 45, 60 and 75 °C. The proportion of cycloadduct in each reaction was determined from an analysis of the proton NMR and the rate of the conversion was determined at each temperature. Using the Arrhenius equation, the enthalpy of activation for the cycloadditions were determined (see Appendix I).

The rates of the loss of bridging carbon dioxide from each of the cycloadducts were investigated at three different temperatures. For cycloadduct **277**, 80, 98 and 115 °C were chosen. The proportion of cyclohexadiene **278** to cycloadduct **277** was determined from the analysis of the proton NMR and the rate of the conversion was determined at each temperature. Using the Arrhenius equation, the activation energy for the loss of carbon dioxide was determined (see Appendix I). For cycloadduct **275**, temperature range of 130, 145 and 160°C were chosen. The proportion of cyclohexadiene **276** to cycloadduct **275** was determined from the analysis of the proton NMR and the rate of the conversion was determined at each temperature. Using the Arrhenius equation, the enthalpy of activation for the loss of carbon dioxide was then determined (see Appendix I). The results of these investigations are shown in (Table 4).

Simultaneously, a computational investigation of the energies of both the transition states ($TS^{\neq}$) for the cycloadditions and loss of carbon dioxide in the reactions of **226** and **227** was carried out. Calculations were performed using Gaussian09.[82] All transition structures were initially optimized with AM1,[83] then reoptimized using B3LYP/6-31G*.[84] This DFT method (model chemistry) has been previously shown by us to be a reliable method for predicting the regio- and stereoselectivity of the cycloadditions of 4-

chloro-2(*H*)-pyran-2-ones.[56] Frequency calculations were then carried out with B3LYP/6-31G(d) and each transition structure was shown to have only one imaginary frequency. Barrier heights were then computed. Constrained optimizations along the route of steepest gradient (IRCs) were then carried out in order to prove that the transition states found connected the minima of interest. The results of these investigations are also shown below (Figure 22 and Table 4).

Table 4

	Energy (calculated)*	**ΔE_A(experimental)**
cycloaddition of 3-carbomethoxypyran-2-one and BVE	19.1 (1.7) Kcalmol^{-1}	8.4 (2.0) Kcalmol^{-1}
cycloaddition of ethyl coumalate and BVE	17.4 (0.0) Kcalmol^{-1}	6.4 (0.0) Kcalmol^{-1}
loss of CO_2 from the cycloadduct of 3-carbamethoxypyran-2one and BVE	25.9 (0.0) Kcalmol^{-1}	21.5 (0.0) Kcalmol^{-1}
loss of CO_2 from the cycloadduct of ethyl coumalate and BVE	28.7 (2.8) Kcalmol^{-1}	26.3 (4.8) Kcalmol^{-1}
*At 273K (sum of electronic energy and thermal energy)		

As can be seen from Table 4, there is a correlation between the experimental and the calculated values. According to calculations, cycloaddition of 3-carbomethoxypyran-2-one and BVE has a 1.7 Kcal/mol higher energy barrier than that for ethyl coumalate and BVE. The energy difference was experimentally measured as 2.0 Kcal/mol. Similarly, the energy barrier for the loss of carbon dioxide from the cycloadduct of 3-3-carbomethoxy-2(*H*)-pyran-2-one and BVE is calculated to be 2.8 Kcal/mol lower than that for the cycloadduct of ethyl coumalate and BVE whilst the activation energy difference is experimentally measured as 4.8 Kcal/mol. Furthermore, both computational and experimental results show that ethyl coumalate **227** is more reactive towards cycloadditions than 3-carbomethoxy-2(*H*)-pyran-2-one **226**; and that the cycloadducts derived from the former are less susceptible to the loss of carbon dioxide than the latter.

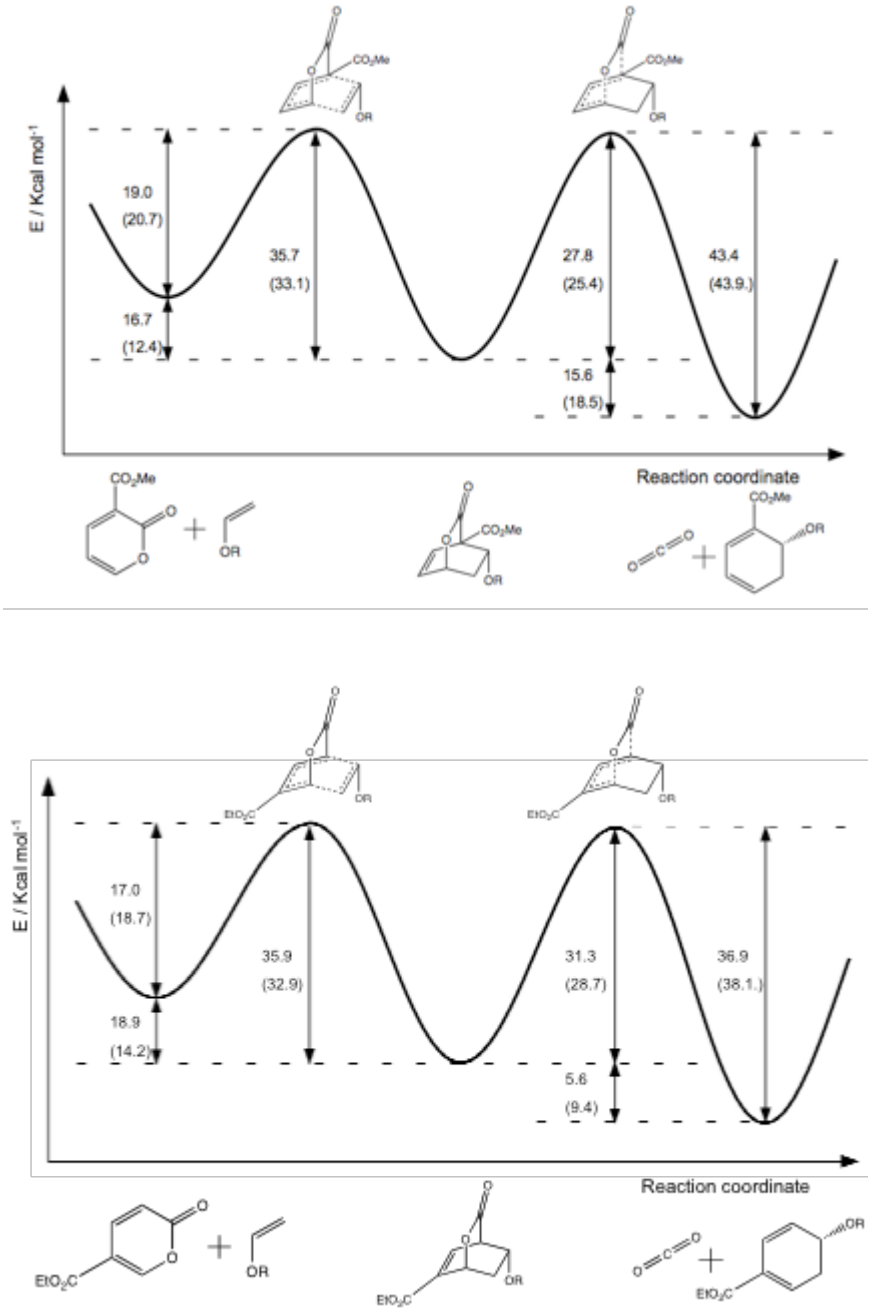

E / Kcal mol-1
19.0
(20.7)
16.7
(12.4)
35.7
(33.1)
27.8
(25.4)
15.6
(18.5)
43.4
(43.9)
Reaction coordinate
CO2Me
OR
EtO2C

Figure 22

2.6.1 Chemical Manipulation of Cyclohexadiene derivative

Until now, the loss of the bridging carbon dioxide from the cycloadducts, although apt for the synthesis of aromatics, had been considered highly disadvantageous for the synthesis of non-aromatic carbocycles, including carbasugars. It clearly removes stereochemical information from the cycloadduct and affords supposedly an unstable cyclohexadiene, which may undergo further reactions. However, the exceptional stability of compound **270a** prompted investigatations into whether this cyclohexadiene can also be manipulated to form a carbasugar.

Although considerable steric information is lost during the retro DA cycloaddition, one clear advantage of using compound **270a** is that there would be no need to carry out a decarboxylation step in the synthesis. At the same time, it was hoped that chirality of the pendant sugar moiety would allow re-introduction of the lost stereochemical features into the molecule.

Therefore, once the identity of compound **270a** was established, the next step was to carry out its conversion to the corresponding pseudodisaccharide **279** (Scheme 74). There were two options: either epoxidation of the C-1/C-2 double bond functionality

and further manipulation of the product, or dihydroxylation of the C-1/C-2 double bond functionality.

Treatment of cyclohexadiene **270a** in DCM/water (3:1 ratio) with *m*CPBA afforded a mixture of epoxides **280a** and **2806b** in a ratio of (2.7:1). Epoxide **280a** was isolated in 63% yield whereas **280b** was isolated in 24% yield (Scheme 75).[85]

The absolute configuration of the major stereoisomer **280a** was confirmed by X-ray crystallography (Figure 23). Presumably, this diastereoselectivity suggests that epoxidation is significantly controlled by intermolecular H-bonding between the peracid and an oxygen atom in one of the many ether linkages contained in the sugar moiety (Figure 24).

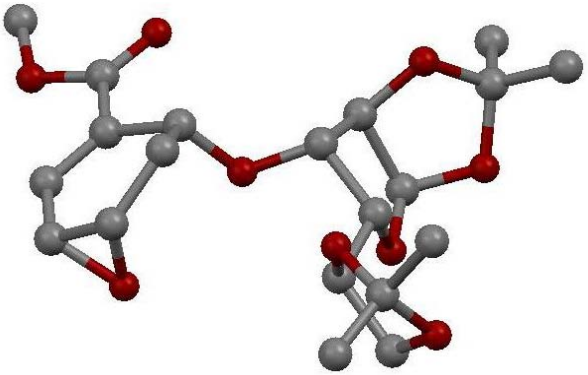


Figure 23: x-ray structure of epoxide **280a**

Figure 24

Treatment of epoxide **280a** in (DME/ethanol/water, 2:1:1 ratio) with sodium azide and ammonium chloride at room temperature for 24 hours resulted in regioselective epoxide opening to afford azide **281** in 85% yield (Scheme 76).[86]

Similarly, treatment of minor epoxide **280b** with sodium azide under similar conditions afforded the corresponding azide **282** in 79% yield (Scheme 77). Raising the temperature of the reaction to 45 ^{o}C improved the reaction yield to 89%.

The remaining steps involve reduction of the azide and the α,β-unsaturated ester and removal of the acetonide protecting groups. It was first attempted to convert azide **281** to the corresponding amine. It was decided to initially investigate selective reduction of the azide using triphenylphosphine and water.[87] (For a mechanism see Scheme 81).

Thus, a solution of azide **281** in DCM was treated with triphenylphosphine and stirred at room temperature for 6 hours before the reaction was quenched with water. However, no desired product was detected. The reaction was repeated at reflux but still no product was detected after quenching with water. Finally, azide **281** was dissolved in THF/water (10:0.1 ratio) and refluxed for 5 hours and again no reaction was observed.

It was concluded that the lack of reactivity of **281** toward triphenylphosphine may be a result of a facile elimination of azide under the reaction condition. Therefore, it was decided to protect the hydroxyl group at C-2 of the cyclohexene/carbasugar ring.

Treatment of azide **281** in DCM with imidazole and TBDMSCl afforded the corresponding silylether **283** in 79% yield (Scheme 78).

Interestingly, the formation of the silylether led to some significant changes in coupling constants in the ^{1}H NMR spectrum (Table 5). These changes were attributed to a comformational change in the cyclohexene ring prompted by the bulky *tert*-butyldimethylsilyl group (Scheme 79).

Table 5

Compound:	$J_{4,3ax}$	$J_{4,3aq}$	$J_{3eq,3ax}$	$J_{2,3ax}$	$J_{2,3eq}$	$J_{1,2}$
281	1.55	3.50	15.46	3.44	2.50	-
283	3.50	9.23	12.72	3.78	12.2	8.25

Refluxing silylether azide **283** in THF/water (10:0.1 ratio) and triphenylphospine **285** for 5 hours indeed afforded corresponding amine **284** in very good yield (90%) (Scheme 80). In this reaction the azide reacts with triphenylphosphine **285** to generate phosphazide **286**, which will lose nitrogen to form iminophosphorane **287**. Then the reaction of water with iminophosphorane **287** gives the corresponding amine **284** and triphenylphosphine oxide **288** (Scheme 81).

Conversion of the ester in **284** to the corresponding allyl alcohol was attempted by treating **284** in dry toluene with DIBAL-H with stirring at -78 ^{o}C for 5 hours. TLC analysis showed that the starting material was consumed, however no desired product could be isolated. It is thought that the ester group was first reduced to the corresponding aldehyde **289**, which consequently reacted with the amine functional group causing polymerisation (Scheme 82).

It was decided to alter the sequence of the reactions. Treatment of **283** with DIBAL-H at -78 ^{o}C afforded the corresponding alcohol **290** in 82% yield without reacting with the azide functional group (Scheme 83). Although the lack of reactivity of the azide with DIBAL-H was surprising, it is not without precedence.[88]

Then azide **290** was reduced using palladium on charcoal (10% weight) to afford the corresponding amine **291** in quantitative yield (Scheme 84).

Once the protected pseudodisaccharide **291** was in hand, a stepwise deprotection of the acetonide and silyl ether protecting groups was carried out by first treating **291** with 90% acetic acid and stirring at room temperature for 18 hours. The acetonide protecting group was not affected even after a longer period of time (30 hours). Normally acetonide groups at C-5' and C-6' are easier to remove and 90% acetic acid should have been sufficient to remove it. A one-pot deprotection was attempted by treating **291** with TFA/THF/water (3:1:0.1) ratio and stirred at room temperature for 24 hours. The crude ^{1}H NMR of the product revealed that all the protecting groups (acetonide and silyl ether) had been removed. Low resolution mass spectroscopy also confirmed that the product was indeed the target pseudodisaccharide **279**. However, due to the polarity of the compound, its chromatographic purification was unsuccessful. It was attempted to acetylate isolated product **279** by treating with acetic anhydride, pyridine and DMAP, however no product could be isolated after chromatographic purification.

It was then decided to alter the sequence of the reactions and carry out the deprotection of the azide **290** followed by reduction. Since the azide functionality is less polar, it was hoped this may help in the purification process. It was planned to carry out a stepwise deprotection by first removing the acetonide group at C-5' and C-6' and the silyl ether protecting group by treating **290** with a mixture of acetic acid/water/TFA (9:1:0.5) and

stirring at room temperature for 4 hours. Indeed the acetonide group at C-5'and C-6' was successfully removed but the silyl ether was still intact. The crude product was purified by flash chromatography to afford **292** in 92% yield (Scheme 85). It was then attempted to remove the remaining protecting groups by treating with acetic acid/water/TFA (7:2:1) but unfortunately, no product could be isolated.

2.6.1.1 Palladium on carbon reduction of Azide 281

The palladium catalysed reduction of azide/alcohol **281** initially gave the corresponding amine **293** but unfortunately amine **293** was very unstable and quickly underwent further reaction. After chromatography, only the sugar part of the molecule was recovered, which was presumably obtained *via* an elimination reaction (Scheme 86).

Similarly when azide/alcohol **282** was hydrogenated in the presence of palladium on charcoal, no product could be isolated. It was assumed that sugar elimination happened in this case.

On the other hand when the silyl ether protected azide **283** was hydrogenated, the corresponding amine **294** was obtained in quantitative yield (Scheme 87).

The difference in the two reactions may be an extension of the observed conformational "flip" which was observed as a result of the conversion of the alcohol function in **281** to silyl ether **283** (Scheme 79).

2.6.1.2 Click Reaction

The azide function is not only a precursor to the amine function but has a diverse and rich chemistry in its own right. In particular, the azide function is shown to be an excellent group for linking different moieties *via* a "click" reaction. Brief investigations into the reaction of azide **283** with phenyl acetylene **295** by means of "click" chemistry. Heating **283** with phenyl acetylene **295** at 80 ^{o}C in the presence of copper(II) sulfate and sodium ascorbate in *tert*-butyl alcohol and water afforded triazole **296** in 99% yield

(Scheme 88). Although there was not time to further investigate the reaction, it is hoped that at a later stage, this reaction can be used to attach this pseudodisaccharide to other molecules including other sugars, fluorescent probes etc

2.7 Conclusion

It has been shown that appropriately substituted pyran-2-ones undergo a facile reaction with vinylated sugars and that the cycloaddition can be used as the basis of a method to prepare carbasugar-"true" sugar pseudodisaccharides. In this method, a free hydroxyl function of a "true" sugar is derivatised to enable attachment of a carbasugar. Stereoselective transformations allow the synthesis of a range of configurations at the newly constructed carbasugar moiety. Investigations into the cycloaddition/cycloreversion reactions of pyran-2-ones were carried out as well exploration into its use in the synthesis of pseudodisaccharides.

Chapter Three: Inhibitors of Golgi α-Mannosidase II

3.1 Introduction

3.1.1 What is Cancer?

Cancer is the second deadliest disease in the developed world.[89] Cancer has been known to practitioners of medicine since antiquity. However its prevalence has only been recognised as infectious diseases, which used to kill humans in large numbers, are being eradicated due to advances in medical science and hygiene.[90]

Cancer is a complex disease both in terms of its etiology and prognosis.[91] Roughly speaking, cancer can be defined as uncontrolled cell division (proliferation),[92] but in fact rapid cell division on its own does not constitute cancer. All cells are genetically programmed to differentiate and proliferate at some stage in their growth.[93] Indeed cell division occurs in nearly all organs and is essential for maintenance of all eukaryotic life. All cells are also genetically programmed to self-destruct if they are damaged, surplus to requirement, or put together in a way that they can not perform their function.[94] This process is known as 'programmed cell death' or PCD, the most prevalent mechanism of which is apoptosis.[95]

In cancer, a cell's normal life cycle is interrupted at one or more stages of its growth. This can be manifested in rapid and uncontrolled cell growth or a lack of PCD, or both.[96] This means that there can be multiple causes for cancer development.

There are two kinds of tumours, benign and malignant.[90] In benign tumour cell growth is localised. Benign tumours are only dangerous if they damage the tissue they are growing in by their mass effect or using too much energy.[90] They are usually treated by

surgical intervention or radiotherapy, unless they are inaccessible.[89] For example, a tumour near the cerebellum is almost always fatal. In malignant tumour, diseased cells either attack healthy cells in the tissue they reside in, or invade those in other tissues.[90] The migration is known as metastasis and is a key event in the pathology of cancer. Cells can change from benign to malignant or vice versa (which is known as remission).[89]

3.1.2 Causes of Cancer

The search for the causes of cancer goes back for centuries. Scientists used to hold different opinions about the real cause of cancer. It is now widely agreed that there are many factors that contribute to cancer. These factors include genetics, smoking, diet, infectious agents (bacteria and virus) and the environment.[97]

From a biological point view, cancer results from either a genetic mutation or a mistranslation of the cell's genetic code.[89] It is important to note that the damage to the cell usually manifests in more than one way. In other words a single 'wrong' protein may or may not cause cancer, but usually more than one kind of 'wrong' protein is produced and the cell may be damaged in more than one way. If PCD mechanisms are not interrupted, the cell dies and cancer does not arise. However, if PCD is not operating the cell will continue to exist and divide. In some cancers, the process of the cell division may also be accelerated concurrently.

The causes of cancer are also varied.[90] Although genetic mutation can be spontaneous, there are a number of mechanisms in the cell for its repair. Any agent that damages deoxyribonucleic acid (DNA)[98] can cause cancer but external agents such as viruses,

bacteria, radiation and carcinogenic chemicals can also initiate cancer by causing a mistranslation of the DNA message.[90,97]

3.1.3 Strategies for Combating Cancer

Cancer is treated in a number of different ways depending on the type of cancer, its tendency to spread and its location in the body.[89] If the tumour is accessible, then surgery is an option.[90] Radiotherapy is an alternative treatment and is used as a means of local control.

Chemotherapeutic agents are another option and are mainly designed to damage the cell's replication process; this can be done by intervention in a number of biological processes.[99] The majority of chemotherapeutic agents currently used in the clinic either target DNA or inhibit the enzymes involved in DNA synthesis and repair. There is another type of chemotherapy which affects signal transduction. Instead of direct attack of the DNA, new generations of anti-cancer drug affect the signals which regulate or promote growth factors and their receptors and cell cycle.[90] There are other strategies employed for combating the disease, these include affecting proteolytic enzyme activity, cell adhesion and angiogenesis in order to slow down cancer disease progression, or conjugating chemotherapeutic agents to tumour cell recognising antibodies.[90]

There is a disadvantage in the use of chemotherapeutic agents. Cancer cells are not the only cells which are dividing and growing. Other cells in the body proliferate; especially bone marrow, epithelial cells in the gut and skin and hair follicles. Chemotherapy will affect these cells and consequently cause unwanted side effects of nausea, vomiting, hair loss and bone marrow suppression.[89]

Another limiting factor of therapeutic agents is the ability of some tumour cells to develop resistance mechanisms against cancer drugs. They do so, either by releasing enzymes which will reverse the cytotoxic and cytostatic effect of the drug or by pumping the drug agents out of the tumour cell.[100]

To reduce side effects and cell resistance to chemotherapeutic agents, it has become important to search for new drugs which will have high selectivity in terms of targeting cancerous cells, thereby affecting normal cells less.

One of the ways to achieve that goal is to inhibit the enzymes that edit cell surface glycoproteins.[100]

3.2 Glycoprotein

The cell is the smallest structure of the body and the fundamental unit of life. The surface of all cells is a complex landscape containing many molecules which are responsible for the interaction between the cell and its environment. One such group of molecules are glycoproteins. Cell surface glycoproteins consist of a cell-surface-embedded protein covalently bound to a polysaccharide projected outside the cell into the extra-cellular matrix (Figure 25).[101]

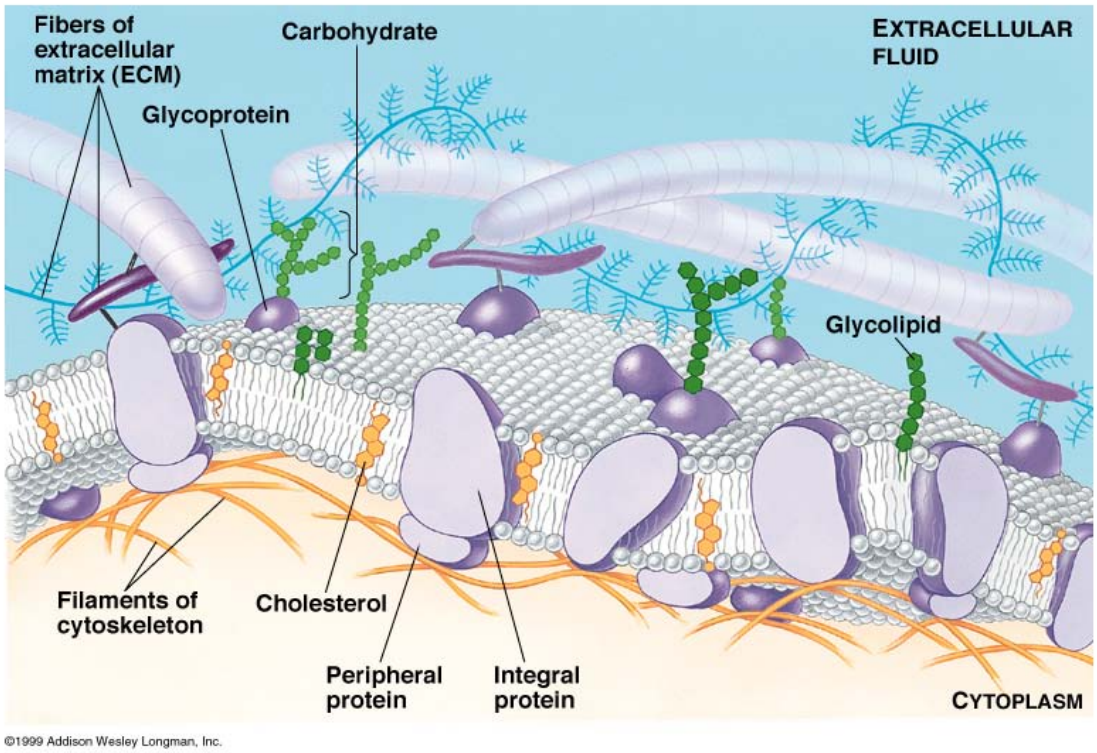


Figure 25: Cell Surface Membrane (Picture taken from http://kentsimmons.uwinnipeg.ca/cm 1504/plasmamembrane.htm)

There are two kinds of glycoproteins depending on the location of the covalent linkage between the protein and polysaccharide. In *O*-linked glycoproteins, the polysaccharide is covalently bonded at its anomeric position to a serine or tyrosine residue of the cell-surface protein[102]. Blood group determinant antigens on the red blood cells are of this type.[103] In *N*-linked glycoproteins, the linkage is through the nitrogen of an asparagine residue.[15] Interestingly, in all eukaryotic cells, although there is a great variety in the polysaccharide component of all cell surface glycoproteins, they all contain a preserved core of five carbohydrate units at their polysaccharide component.[102]

A detailed study of the biosynthesis of cell surface glycoproteins over the past 20 years has shown that all of them are made by addition/cleavage of carbohydrate units to that core. The glycosylation mechanisms (addition of sugars) of the two classes of

glycoproteins are different.[104] We are interested in the synthesis of *N*-linked glycoproteins and we will explore it in more detail.

Asparagine-linked (*N*-linked) glycoprotein biosynthesis is a complex process, occurring in the membranes of the secretory pathways of the endoplasmic reticulum, Golgi apparatus, trans-Golgi network and transport vesicles between these organelles.[105] The protein backbone is first synthesised on ribosomes and is then glycosylated. The synthesis starts with oligosaccharide maturation which occurs in four stages.[105] The first stage happens on the cytoplasmic phase of the endoplasmic reticulum and involves the synthesis of a lipid-linked precursor oligosaccharide.[106] The second stage involves the transfer of the lipid-linked precursor to a newly synthesised peptide forming a linkage to an Asn residue.[105] Trimming *N*-glycans occur in the third stage and involve the removal of a variable number of mannose and three glucose residues. At this stage, α-glucosidase and α-mannosidase remove four of the original nine mannose units to generate $Man_5GlcNAc_2$-N which is then added to GlcNAc to create the $GlcNAcMan_3GlcNAc_2$-N intermediate.[107] The final step of the maturation process is the extension and branching of the oligosaccharide, which results in the final glycosylated structure which is transported to destinations such as the cytoplasm, lysosomes and plasma membrane.[108] The glycoprotein glycosylation is best illustrated in Figure 26.[105]

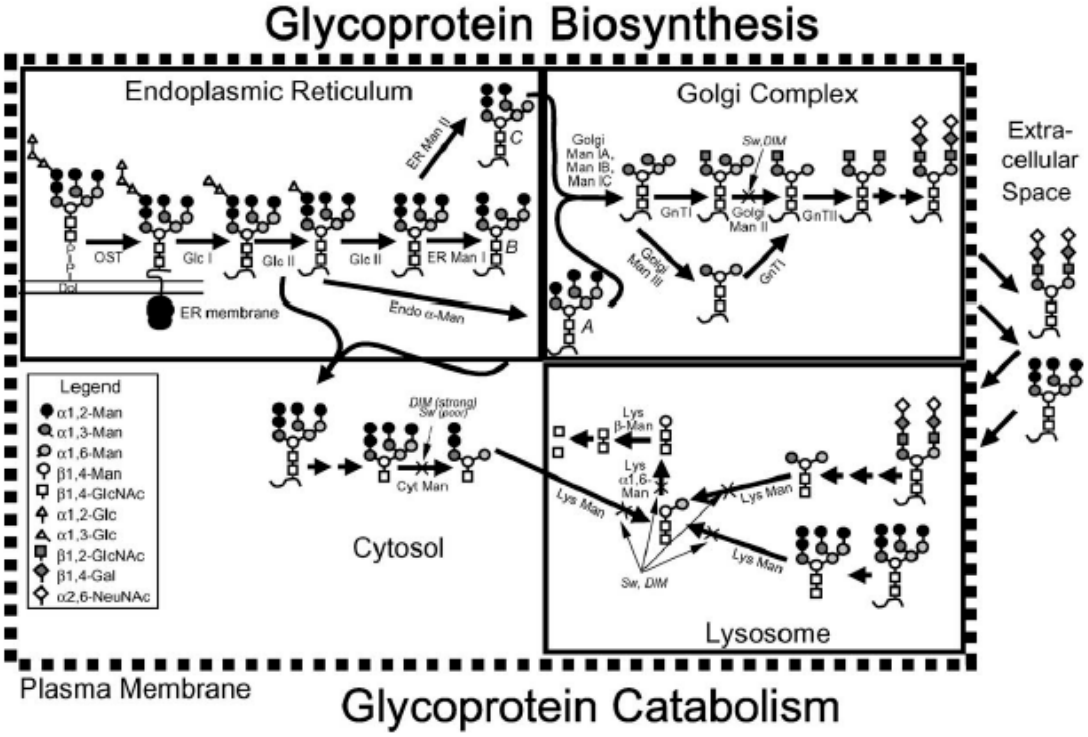
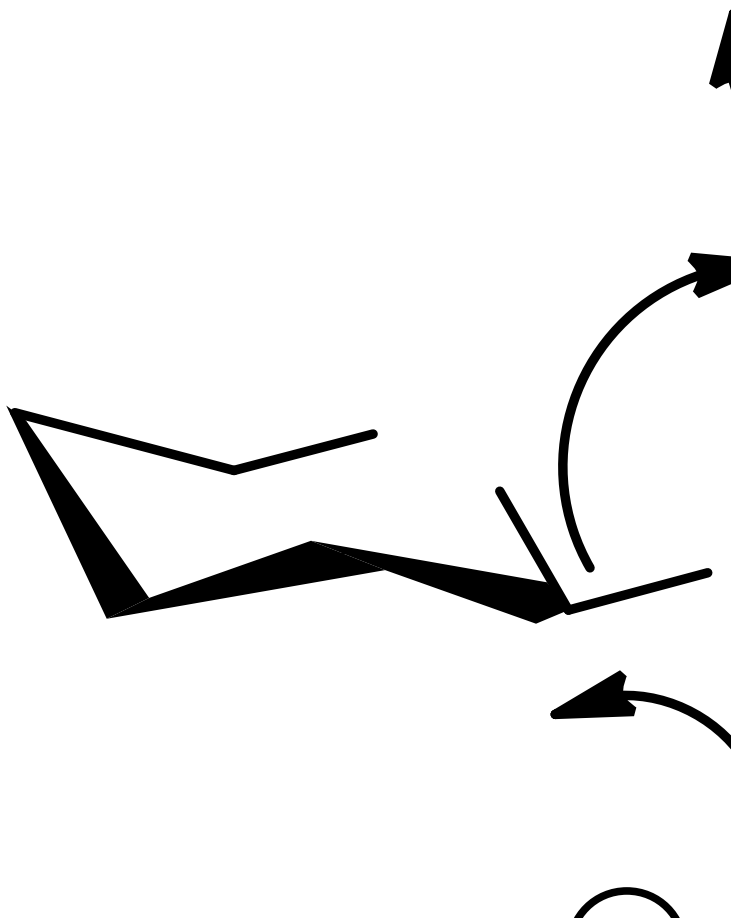


Figure 26: *N*-linked glycoprotein processing. (Picture taken from *Biochim. Biophys. Acta*, **2002**, *1573*, 225-235).

3.2.1 Glycolysis Mechanism

General enzymatic hydrolysis of the glycosidic bond normally takes place via an acid-base reaction which requires a nucleophile and a proton acceptor.[109] There are two possible stereochemical outcomes for the hydrolysis of such a bond: retention and inversion of configuration (Scheme 89).[110] In the transition state, an oxocarbenium ion is formed which is held in between two carboxylic acids at the active site of the enzyme.[110] In retention glycosidases (Path a), the hydrolysis occurs *via* a double displacement mechanism where the two carboxylic acids are located 5.5 Å apart. In contrast, in the inverting transition (Path b), the two carboxylic acid residues are 10.5Å apart and the reaction proceeds via a single displacement mechanism.[110]



The mechanistic understanding of the details of glycosidase-mediated hydrolysis is important for the discovery and development of stronger and more selective inhibitors that would have therapeutic potential.[111] Relenza **297**, a sialidase inhibitor for treatment of influenza, and acarbose **10**, an α-amalyse inhibitor for control of blood glucose, are examples of glycosidase inhibitor based therapeutic agents developed in recent years (Figure 27).[7,110]

Figure 27

3.3 Golgi α-Mannosidase II Inhibition as a Cancer Therapy

One of the important enzymes in late stage glycoprotein processing is Golgi α-mannosidase II (GMII).[112] The enzyme is involved in the catalytic process of trimming two mannose residues from intermediate **298** to give glycoprotein precursor **299** (Scheme 92)[113]. In cancerous cells, altered distribution of glycoprotein (increased branching) is observed and is associated with disease progression and metastasis.[114] The inhibition of α-mannosidase II is a potential anti-cancer strategy.[113,115]

Processing α-mannosidases can be divided into two classes, each with distinct biochemical properties.[116] Class I α-mannosidases, inverting glycosidases, cleave only α-1,2-mannose residues.[117] They are inhibited by pyranose mannosaccharide analogues such as 1-deoxymannojirimycin **300**. Class II α-mannosidases, retaining glycosidases, cleave α-1,2-, α-1,3-, and α-1,6-mannose residues. They are inhibited by furanose analogues such as swainsonine **301** (Figure 28).[117] Since α-mannosidase II is a retaining enzyme, the hydrolysis it catalyses will occur *via* a double displacement mechanism.

Figure 28

Swainsonine **300** is an indolizidine alkaloid natural product found in North American plants of genus *Astragalus*, Australian *swainsona canscens* and the fungus *Rhizoctonia leguminicola*.[118] As inhibitors of α-mannosidase II it has a potential anticancer chemotherapeutic application.[113,118]

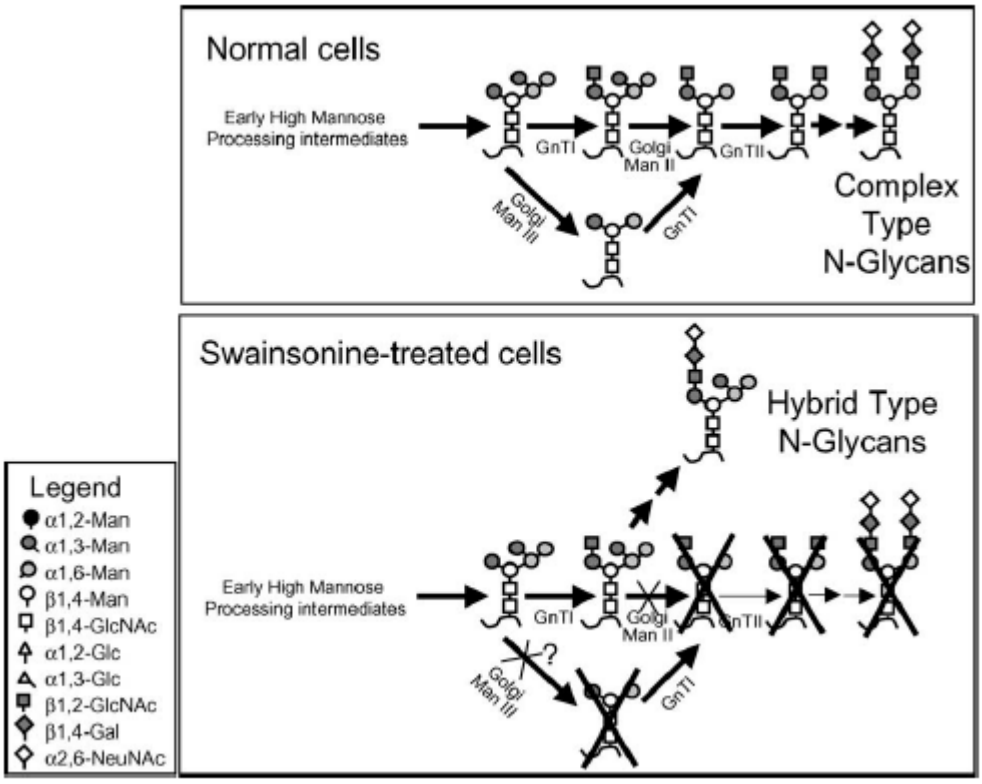


Figure 29: The effect of swainsonine on cells. (Picture taken from *Biochim. Biophys. Acta*, **2002**, *1573*, 225-235).

Swainsonine was originally used in research as a means to investigate and determine whether alterations in *N*-linked oligosaccharide structures affect the function or intracellular processing of glycoproteins in biological systems.[119] It was shown that when a swainsonine solution is injected into a cultured medium of animal cells, *N*-linked oligosaccharides produced have a hybrid structure instead of a complex one, an observation which is consistent with mannosidase II enzyme inhibition[120] (Figure 29). In other words, swainsonine inhibits α-mannosidase II, but not α-mannosidase I, and

protein glycosylation proceeds up to the formation of $Man_5GlcNAc_2$, after which the pathway is diverted to form hybrid-type *N*-glycans.[112]

Swainsonine **301** has been synthesised through a number of different routes.[121] An exemplar synthesis of swainsonine from α-furyl amide **302** is shown in Scheme 91.[122]

Swainsonine had shown several advantages over other glycosylase inhibitors, including low preclinical toxicity.[112] *In vivo* studies on swainsonine **301** were carried out on mice and showed that it reduces metastasis, solid tumour growth and enhances antitumour immune response in mice.[123] Specifically, it stimulates natural killer cells, lymphokine activated killer cells and anti-tumour activity of macrophages.[105] In a phase II clinical trial study in patients with advanced malignancies, administration of swainsonine reduced tumour growth and metastasis is observed.[112,123] Swainsonine also showed low toxicity in humans in early trials.[105]

However, swainsonine has been subsequently associated with serious side effects, namely its co-inhibition of lysosomal mannosidase II, which causes the accumulation of mannose in tissues[100,112,113] For this reason, it became important to search for more selective α-mannosidase II inhibitors.[100]

Good α-mannosidase II inhibitors must satisfy a number of conditions in order to become potential drugs. These conditions include, low toxicity, enzyme specificity and membrane permeability.[124] Inhibitors are made membrane permeable by introducing lipophilic groups to inhibitor molecules.

Figure 30

Pearson and co-workers have reported swainsonine analogues with interesting inhibitory properties.[113] Since swainsonine does not discriminate between GM II and lysosomal α-mannosidase II, they tried to make selective inhibitors which would inhibit GM II but not lysosomal α-mannosidase II.[113] Their hypothesis was based upon adding a sugar or sugar-like molecule to swainsonine **301** at C(3), thereby yielding a selective Golgi mannosidase II inhibitor. They suggested that the GM II catalysed hydrolysis transition state involves mannosyl cation **313** and the C(3) position of swainsonine **301** would correspond to the anomeric carbon of **313** (Figure 30).[113] Indeed, some of the analogues they have developed exceeded swainsonine in their ability to inhibit jack bean α-mannosidase II. For example, analogues **314** and **315** gave IC_{50}'s of 50 and 70 nM respectively compared to compound **301** which gave (400 nM).[113]

Swainsonine analogues **314** and **315** are synthesised from D-ribose **316** as shown in Scheme 92.[113,125]

Other α-mannosidase inhibitors include mannostatin **320**[126], salacinol **321**[127], D-glycon-δ-lactams such as **322**[128], compound **323**[129], five-membered ring amino sugar **324**[130], 5-thio-D-glycopyranosylamines **325** [131] and α-D-glucopyranosyl-(1→3)-1-deoxymannojirimycin **326** (Figure 31).[132,133]

Figure 31

3.4 Conclusion

The search for selective GM II inhibitors is an evolving and promising area of cancer research. Obtaining exact information about glycosidase mechanisms will help scientists in designing potent inhibitors.

3.5 Results & Discussions

3.5.1 Objectives

Figure 32

Our approach in the search for new inhibitors of Golgi α-mannosidase II is based on the synthesis of 6-aminosubstituted mannose derivatives **327** (Figure 32) and determination of their inhibitory potential towards commercially available jack bean α-mannosidase II. The design of **327** as an inhibitor is based on two principles. Firstly, by using a non-anomeric linkage in **327**, the molecule can bind to the enzyme but not be turned over, thus acting as an inhibitor. Secondly, by replacing a nitrogen atom for the oxygen atom at the exocyclic anomeric position, we hoped for an increase in binding of **327** to the enzyme since the proposed mechanism for the cleavage by GMII (Scheme 91) requires protonation of this heteroatom. Since the nitrogen atom is a more basic atom than oxygen, **327** should interact more strongly with the enzyme. Since the R group in **327** is

supposed to mimic a sugar, we proposed that the most suitable groups would be cyclohexyl or benzyl groups with hydroxyl or methoxy substituents, e.g. **328** and **329**.

The original retrosynthetic analysis of compound **328/329** is shown in Scheme 93. We envisaged that **328/329** can be prepared *via* a reductive amination of protected aldehyde **331** and an amine **330**. Aldehyde **331** in turn can be prepared from the protected mannose derivative **333** which can be easily prepared from commercially available methylmannopyranoside **334**.

3.5.2 Synthesis of 6-substituted mannose derivatives[134]

We started our synthesis with commercially available α-D-methylmannopyranoside **334**. The primary hydroxyl group in α-D-methylmannopyranoside can be protected selectively since it is more reactive than the rest of hydroxyls on steric grounds. This was achieved by treating **334** with TBDMSCl in pyridine, in the presence of a catalytic amount of DMAP to afford 52% yield of product **335** (Scheme 94). DMAP is a nucleophilic catalyst as it displaces the chloride in the TBDMSCl to give **336** *in situ*. DMAP is a better leaving group than a chloride ion as it forms a neutral species once it is eliminated. The full mechanism of the reaction is shown in Scheme 95.

The remaining hydroxyl groups in compound **335** were protected with benzyl groups. This was achieved by treating the 6-silyloxy mannoside **335** with sodium hydride in DMF with a catalytic amount of TBAI before the addition of BnBr. The iodide in TBAI is a nucleophilic catalyst as it will replace the bromide in benzyl bromide to give benzyl iodide, which is a better alkylating agent compared to benzyl bromide as iodide is a better leaving group than bromide. The benzylation proceeds with deprotonation of the

hydroxyl groups to generate alkoxides which in turn act as nucleophiles to attack benzyl iodide as shown in the Scheme 97.

The fully protected mannoside **337** was desilylated under mildly acidic conditions in MeOH to give primary alcohol **333** (Scheme 98). The mechanism of the hydrolysis proceeds via protonation of the oxygen on C-6, followed by nucleophilic attack of water on silicon to displace the protonated primary alcohol. The final step of the reaction is loss of a proton to give silyl product **338** (Scheme 99).

We next attempted to oxidise the primary alcohol in **333**, by treating with pyridinium chlorochromate (PCC) in DCM as reported in the literature.[134] Unfortunately no product could be isolated. This prompted us to use other methods for oxidising primary alcohols to aldehydes, e.g. the Swern oxidation. In the course of this method, the primary alcohol was treated with oxalyl chloride in DCM, followed by DMSO and triethylamine. However, this method also did not yield any product.

For this reason, we decided to revise our strategy and we came up with a new route to synthesise our target molecules. The new retrosynthetic analysis is shown in (Scheme 100).

We converted primary alcohol **333** to tosylate **341** by treating **333** in pyridine with tosyl chloride to afford **341** in 88% yield. Heating **341** with sodium azide at 90 ºC for 4 hours afforded azide **342** in good yield (97%). Azide **342** was then reduced to the corresponding amine in 72% yield (Scheme 101).

Primary amine **339** was then coupled with a number of aryl aldehydes and cyclohexanone derivatives. First the amine, together with the corresponding aldehyde or ketone was heated under reflux for 24 hours and then the resulting imine was reduced using sodium borohydride (Scheme 102). The results are summarised in Table 6.

The benzyl protecting groups of compound **343** were removed by catalytic hydrogenation over palladium on charcoal in methanol over 3 days to afford **351** (Scheme 103).

Table 6: Coupling amine **339** with aldehyde/ketone

Entry	Aldehyde/ketone	Product	Yield (%)
1			56
2			89
3			83
4			61

However, when compound **344** was hydrogenated using palladium on charcoal in methanol overnight, amine **339** was re-formed. Surprisingly, instead of cleavage of the *O*-benzyl groups, hydrogenation preferentially removed the *N*-benzyl group (Scheme 104).

This prompted us to rethink our synthesis strategy again. We decided this time to work with unprotected sugars, since protecting and then de-protecting sugars is a long method and sometimes not reliable as we have experienced.

We started our synthesis with α-D-methylmannopyranoside **334** and tosylated the 6-hydroxyl group with tosyl chloride, followed by nucleophilic attack with sodium azide to furnish 6-azido mannose **353** in 96% yield. 6-Azido mannose **353** was then reduced to the corresponding amine **354** in 72% yield (Scheme 105).[135]

We then carried out a series of reductive aminations between 6-amino mannose **354** and a number of aryl aldehydes and cyclohexanone derivatives. First the amine and aldehyde or ketone were heated under reflux for 24 hours and then reduced using excess sodium borohydride to yield the desired product **355** (Scheme 106). The results are summarised in Table 7

Table 7: Coupling amine **354** with aldehyde/ketone

Entry	Aldehyde/ketone	Product	Yield (%)
1			63
2			59
3			53
4			49

3.5.3 Biological activity

The compounds were screened in an *in vitro* enzyme inhibition assay against Jack bean α-mannosidase II using a protocol supplied by Vasalla. In this test, inhibition of the enzyme is measured as a reduction in the rate of hydrolysis of *p*-nitrophenyl-α-D-mannopyranoside in the presence of the inhibitor. Unfortunately, all compounds were found to lack inhibitory action. Detailed procedure of the assay is provided in the experimental section.

3.5.4 Conclusion

We have successfully synthesized 5 target molecules as potential inhibitors of GM II. We evaluated their inhibition against jack bean α-mannosidase, and the compounds were not active.

Chapter Four: 6-Anhydro-(1,2,3-triazolo)sugars as inhibitors of glucokinase

4.1 Introduction

4.1.1 Glycolysis

Glucose **215** is the major source of energy for almost all organisms. In the human body, glucose is the only source of energy for the brain and red blood cells. Every cell in the body is able to generate adenosine triphosphate (ATP) from glycolysis, a sequence of reactions that converts glucose to pyruvate. If this process is anaerobic (i.e. occurring in the presence of low or zero levels of oxygen), the product of glycolysis, pyruvate, is further processed to lactate (in higher animals) or ethanol (in yeast). Under aerobic conditions on the other hand, pyruvate can be completely oxidised to carbon dioxide *via* tricarboxylic acid TCA (Kreb's) cycle releasing even more ATP.

The glycolysis process takes place in three stages.[136]

Stage 1: Conversion of glucose into fructose-1,6-bisphosphate

This process consists of three steps: in the first step, glucose is phosphorylated to give glucose-6-phosphate (G6P) **360**. This reaction is mediated by the enzyme *hexokinase*. Glucose 6-phosphate **360** is both a product and an inhibitor of hexokinase which means its cellular accumulation is prevented under normal conditions. The second step involves isomerisation of glucose 6-phosphate **360** into fructose 6-phosphate **361** by the action of phosphoglucose isomerase. The final step is the phosphorylation of fructose 6-phosphate **361** to yield fructose-1,6-bisphosphate **362**. Although energy will be generated in later stages of glycolysis, reactions in this first stage consume energy particularly the first and third steps (Scheme 107).[136,137]

Stage 2: Cleavage of fructose-1,6-bisphosphate to two triose phosphates

Fructose 1,6-bisphosphate **362** is cleaved into glyceraldehydes-3-phosphate **363** and dihydroxyacetone phosphate **364** (Scheme 108). The two products are isomers; one is an aldehyde while the other is a ketone. They are readily interconverted by *triose phosphate isomerase*.[136,137]

Stage 3: Energy harvesting

This stage consists of a series of steps in which ATP is generated. Glyceraldehyde 3-phosphate **364** is converted into 1,3-bisphosphoglycerate **365** by the action of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). 2 molecules of ATP are generated from ADP when 1,3-bisphosphoglycerate **365** is converted into 3-phosphoglycerate **366**. This reaction is catalysed by phosphoglycerate kinase (PGK). A further 2 molecules of ATP are released when two molecules of 1,3-biphosphoglycerate are converted into two molecules of pyruvate **369** (Scheme 109). Overall, glycolysis generates a net 2 moles of ATP (as well as 2 moles of NADH and 2 moles of pyruvate) per mole of glucose **215**.[136,137]

Pyruvate **369** is the end product of glycolysis and is converted to acetyl–CoA by pyruvate dehydrogenase before it enters the TCA cycle.[138] During this stage of metabolism, each mole of pyruvate generates a further 4 moles of NADH, a mole of GTP and a mole of $FADH_2$.

In general glycolysis is a well regulated pathway due to the necessity to respond to conditions inside and outside of the cell.[139]

4.1.2 Lactate metabolism

Lactate is generated from pyruvate when there is a great need for energy but the amount of oxygen in the cells is limited and so further oxidation of pyruvate to carbon dioxide is not possible. This process which releases a further small amount of ATP is catalysed by lactate dehydrogenase (LDH).[139] Lactate metabolism is common in muscle cells during strenuous exercise when the demand for energy is very high. The resulting lactate is transported out of the cell and into the blood to highly aerobic tissues such as the heart, where lactate is either converted back to glucose or catabolised further through aerobic respiration.

4.1.3 Cancer and Glycolysis

An increase in metabolic activity is associated with fast growing cancer cells. Since the microenvironment around many tumour cells is oxygen deficient (hypoxic), non-aerobic metabolic pathways become very important to cancer cells as a means of producing the energy they need to survive and grow.

Cells in embryonic development and those in exercising muscles experience hypoxia because the demand exceeds the supply of oxygen *via* the cardiovascular system. In cancer, tumour cells out-grow the vascular systems that would supply them with oxygen. To gain enough energy to survive and replicate**,** cancer cells inside hypoxic tumours make glycolysis more efficient by the action of a transcription factor known as hypoxic

inducible transcription factor (HIF-1).[140] Formed when oxygen is not present in the cell environment, HIF-1 increases the expression of glucose transporters and glycolytic enzymes. In this way, the cell can get most of its energy from the anaerobic glycolytic pathway.

For example, HIF-1 induces pyruvate dehydrogenase kinase-1 (PDK1)[141] which inhibits pyruvate dehydrogenase by phosphorylation.[142] This in turn blocks the pyruvate entry into mitochondrial metabolism and hence promotes anaerobic metabolism towards lactate.[143] The resulting lactate is released into the extracellular space and regenerates NAD^+ for continued glycolysis under anaerobic conditions.

Since tumour cells survive and grow under hypoxic conditions, there are several studies investigating how suppression of glycolytic pathway enzymes will affect tumour growth. Fantin *et al* investigated the effect of LDH-A suppression on cancer cell growth, metabolism and tumourigenicity.[144] When LDH-A is suppressed (using expression of short hairpin RNAs complementary to LDH-A) in neu-initiated mammary epithelial cell lines, oxidative phosphorylation is enhanced to compensate for the lack of anaerobic metabolism.[144] They also noticed a decrease in tumourigenic and proliferative potential of the cancer cells.[144]

There are two explanations for these observations: first, oxidative phosphorylation alone cannot adequately sustain the metabolic demands of cancer cells, and secondly, glycolytic metabolites are used for the synthesis of fatty acids and non-essential amino acids necessary for cell growth.[144,145] Therefore suppression of glycolysis results in reduced growth and tumourigenicity of cancer cells.

4.2 Aims of the project

We are synthesising a series of 6-triazole glucose and mannose derivatives as mimics of glucose-6-phosphate (G6P) by employing click chemistry.

4.3 Click chemistry

4.3.1 Introduction

Sharpless and co-workers have described a group of reactions that selectively form heteroatom links called 'click reactions'.[146] In order for a reaction to be useful in this context, it must be modular, high yielding, wide in scope, generate only inoffensive by-products and must have a thermodynamic driving force greater than 20 kcal/mol.[146]

Reactions designated as click chemistry include: cycloaddition reactions (especially hetero-Diels-Alder reactions), nucleophilic ring opening reactions (such as opening of epoxides and aziridines), addition to 'carbon-carbon' multiple bonds and carbonyl chemistry of the non-aldol type.[146]

4.3.2 Copper(I) catalysed azide-alkyne cycloaddition

The best click reaction to date is the Cu^{I}-catalysed Huisgen 1,3-dipolar cycloadditon of azides and alkynes which affords 1,2,3-triazoles. This Cu^{I}-catalysed reaction was reported independently in 2002 by two groups (Sharpless and Madel).[147,148] Since then, it has quickly found many applications in chemistry, biology and material sciences. It

has been used for the synthesis of biologically active molecules,[149] for selective labelling of modified bacterial cell walls[150] and the synthesis of dendrimers.[151]

Uncatalysed alkyne-azide cycloadition is a slow process, normally requiring elevated temperature and the product is a mixture of 1,4- and 1,5-regioisomers. For example, the thermal reaction between benzyl azide **370** and phenyl propargyl ether **371** gave both isomers in a ratio of 1:1.6 in favour of the 1,4 regioisomer **373** (scheme 110). When the same reaction was carried out under click conditions, 1,4-regioisomer **373** was formed exclusively (Scheme 111).[147]

The use of a variety of solvents was reported, including ethanol or *tert*-butanol and even water without an organic co-solvent. The catalyst loading reported is in the range of 0.25 to 2 mole%.[147]

Table 8: Some examples of Cu^{I}-catalysed alkyne-azide couplings from literature.[147]

Entry	Azide	Alkyne	Product	Yield (%)
1				92
2				93
3				82
4				84
5				91
6				88
7				88
8				84

4.3.3 Sources of copper(I) catalyst

The sources of copper(I) catalyst are varied. One of these sources is *in-situ* generation of copper(I) from copper(II) sulfate pentahydrate. This process occurs either by reduction or by conproportionation with copper metal. In the reduction route, sodium ascorbate is used as the favoured reducing agent.[147,151] The ratio of sodium ascorbate to copper used, ranges from 3 to 10 fold. In biological systems tris(caboxyethyl)phosphine is the favoured reducing agent.[152]

Oxidation of copper metal is another source of copper(I) catalyst. This is achieved by direct addition of copper turnings to azide-alkyne reactions in alcohol/water solvents.[153] Nanosize Cu^0 can be used to accelerate the rate of reaction since use of copper turnings in reactions require long reaction times.[154]

On the other hand, copper(I) salts, such as copper(I) iodide, $CuOTf.C_6H_6$ and $[Cu(NCCH_3)_4][PF_6]$, can be used as a copper(I) sources.[147] Normally one equivalent of base and acetonitrile as a co-solvent are needed. This method has the disadvantage of byproduct generation, in the form of bisacetylenes, 5-hydroxytriazoles and bis-triazoles.[147,148] The use of 2,6-lutidine and DIPEA improves the reaction by minimising byproduct formation, and oxygen exclusion further improves the yield and purity of the product.[147]

4.3.4 Mechanism of copper(I) catalysed azide-alkyne reaction

The proposed mechanism begins with copper(I) insertion into a terminal alkyne (no reaction is observed with internal alkynes), and proceeds with stepwise formation of a six-membered copper containing species **379** (Scheme 112).[153]

In mechanistic studies, acetonitrile was used as a ligand for the copper complex as well as the solvent. In the reaction, copper(I) acetylene formation starts with coordination of alkyne **375** with copper(I) species **374** to displace one of the acetonitrile ligands (Scheme 113).[153] In acetonitrile, this step is endothermic by 0.6 kcal/mol. But, in aqueous solutions, the displacement process is exothermic by 11.7 kcal/mol.[153] This is consistent with experimental observations of rate acceleration in aqueous medium. The initial coordination of copper and alkyne to form a π complex lowers the pK_a of the alkyne C-H **382** by up to 9.8 units.[153]

After formation of the copper acetylene complex, the azide displaces one ligand and binds with copper to generate intermediate **378**.[153] This process is slightly exothermic, and is followed by nucleophilic attack of the distal nitrogen of the azide at C(4) of the acetylene, generating six membered copper(III) metallacycle **379**.[153] This is followed by ring contraction which is facilitated by transannular association of N(1) lone pair of electrons with Cu π^* orbital. Transformation of **379** to **380** is fast due to the low energy barrier. Proteolysis of triazolyl-copper derivative **380** forms product **381** and hence regenerates the catalyst.[153]

4.3.5 One-pot multi-step synthesis of triazole linked glycoconjugates

Due to the perceived dangers of handling organic azides, *in situ* generation of azides enhances the appeal of the copper(I) catalysed azide-alkyne reaction. Wang and co-workers reported the one-pot synthesis of triazole substituted glycoconjugates from an unprotected monosaccharide.[155] In this one-pot synthesis, four transformations are achieved without isolating any intermediates. The process begins with acetate protection of the sugar followed by brominolysis and subsequent azide displacement and cycloaddition to afford the desired triazole product in good yield (Scheme 114).[155]

With protected D-glucose and D-galactose as starting materials, the reaction furnished the expected products smoothly at room temperature overnight. Due to the steric

hindrance of the 3-substituent of D-mannose, the rate of the reaction is slow and elevated temperature is needed to obtain the desired triazole product from that sugar.[155]

4.3.6 Limitations of Cu^{I}-catalysed alkyne-azide reactions

Cu^{I}-catalysed alkyne-azide coupling usually generates the desired triazole in an efficient and reliable way. How ever some research groups have reported reactions with low yield due to byproduct formation, or generation of unexpected products.[148,156] In the case of reactions with low yield, alkyne homocoupling is a possible cause. Alkyne homocoupling is a known reaction which is also catalysed by Cu^{I} species and small, unhindered amines promote this conversion through stabilisation of intermediates of alkyne homocoupling **384** and **385** (Scheme 115).[157]

On the other hand, Chang and co-workers reported unexpected high yield of compound **389** from a three component coupling of alkyne **295**, azide **387** and amine **388** under click conditions (Scheme 116).[156] In the absence of Cu^{I} no reaction took place indicating the important role of the copper(I) catalyst.

There are two possible pathways for this reaction. The first possible pathway (A) is the formation of ketenimine intermediate **391** from copper acetylene and azide coupling, followed by amination across the C=N bond and finally tautomerisation to form the imidine **389**.[156] The second pathway (B) involves formation of triazole intermediate **392**, followed by Dimroth rearrangement *via* α-diazoimine and finally reaction with amine generates imidine **389** (Scheme 117).[156]

4.4 Retro-synthetic analysis of the target molecule

The first step of glycolysis is the phosphorylation of glucose to form glucose-6-phosphate (G-6-P). This reaction is catalysed by the hexokinase enzyme (Scheme 118). Glucose 6-phosphate is both a product and an inhibitor of hexokinase. We are trying to design molecules which can mimic G-6-P, so they can inhibit the activity of hexokinase. The molecules we have designed have the triazole functional group substituted at the 6 position on mannose and glucose compounds **393**. Triazole-substituted molecules have been used before as inhibitors of phosphatase. Seto and co-workers have designed triazole substituted compounds as protein tyrosine phosphatase inhibitors.[158] We have synthesised the target molecules by making azide sugars (mannose and glucose) and subsequent cycloadition of azide and alkyne to generate the desired triazole. The retrosynthetic analysis of compound **393** is shown in Scheme 118.

4.4.1 Synthesis of sugar azides (mannose and glucose)

We started our synthesis with commercially available α-D-methylmannopyranoside **334** and α-D-methylglucopyranoside **394**. The 6-hydroxyl group of the saccharide (glucose and mannose) was tosylated with tosyl chloride. The tosylate was then treated with sodium azide to furnish 6-azido saccharides **353** and **396** in good yields (Scheme 119).

4.4.1.1 Thermal coupling of Azide and Alkyne

Azide **353** and methyl propiolate **397** were heated under reflux in methanol for 24 h and afforded a mixture of 1,4- and 1,5-regioisomers of the corresponding triazoles **398** and **399** in a 1:3.7 ratio (Scheme 120).

However, when propargyl alcohol **400** or phenyl acetylene **295** were heated with azide **353**, no product was isolated. Therefore, we decided to use the copper(I) catalysed alkyne-azide coupling to obtain the desired products.

4.4.1.2 Cu^{I}-catalysed Alkyne-Azide coupling

Although a number of copper(I) sources are available, we used copper(II) sulphate pentahydrate, where copper(I) is generated *in situ* when copper sulfate is reduced by sodium ascorbate. The catalyst loading was 1 mol%. Our solvent of choice was *tert*-butyl alcohol with water as the co-solvent in a 1:1 ratio.

The reaction between 6-azido mannose **353** and propargyl alcohol **400** proceeded to completion in 40 h at room temperature (Scheme 121). However, the reaction with phenyl acetylene **295** was very slow. Carrying out the NMR of the crude reaction mixture after 60 hours indicated that much of the starting material was left unreacted.

When the reaction temperature was elevated to 80 ^{o}C, the reaction went to completion in 15 hours. Subsequently, in order to increase the rate of the reaction, all azide-alkyne coupling reactions were carried out at 80 ^{o}C over 15 hours (Scheme 122).

The results of the cycloaddition reactions are summarised in Tables 9 and 10. The coupling of 6-azido mannose and methyl propiolate under click conditions gave only one regioisomer (1,4-isomer), which confirms the regioselectivity of this reaction.

Table 9: Results of Cu^{I}-catalysed 6-azido mannose and alkyne coupling

Entry	Alkyne	Product	Yield (%)
1			70
2			58
3			45
4			76
5			56

Table 10: Results of Cu^{I}-catalysed 6-azido glucose and alkyne coupling

Entry	Alkyne	Product	Yield (%)
1			69
2			84
3			34
4			67

4.4.2 Biological activities

The compounds were screen in an *in vitro* cell survival assay using colorectal HCT116 $^{p53+/+}$ (both p53 alleles intact) and HCT116 $^{p53-/-}$ (both p53 alleles disrupted) to asses their cytotoxic poteantial but were found to be inactive. Detailed procedure is provided in the experimental section.

4.4.3 Conclusion

We have successfully synthesised 6-substituted triazole sugar molecules using click chemistry. Biological assay of these molecules showed that they were inactive.

Chapter Five: Experimental

5.1 General Experimental

Most commercially available reagents were used as received without additional purification. A minority of compounds were additionally purified and dried according to accepted procedures. Products were typically purified by column chromatography using Merck 9385 silica gel 60 (40-63 μm). Analytical thin layer chromatography (TLC) was conducted on Merck silica gel 60 F_{254} glass backed plates. Visualisation of the reaction components was accomplished by illumination under short wavelength (254 nm) ultraviolet light or using basic potassium permanganate ($KMnO_4$) stain.

Proton Nuclear Magnetic Resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ and MeOD using spectrometers operating at 360, 600 MHz. Carbon Nuclear Magnetic Resonance (^{13}C NMR) were performed on the same instruments operating at 101 and 150 MHz respectively. Chemical shifts are relative to an internal standard (tetramethylsilane, $\delta = 0.0$) and are reported in ppm. Assignments of the NMR spectra were confirmed by COSY, DEPT and HMQC. Sodium chloride plates were used to run the IR spectra. Solids were run as a dispersion in nujol or by grinding with potassium bromide powder, and oils were run neat as a thin film. Electrospray ionisation (ESI) technique was used for low resolution and high resolution mass spectroscopy. Petroleum ether (petrol) refers to the 60-80 ˚C boiling point fraction. Optical rotations were measured with a Perkin Elmer polarimeter (Model 341).

1,2,:5,6-Di-*O*-isopropylidene-α-D-glucofuranose **216**

Sulphuric acid (98%, 16 mL) was added dropwise over 15 mins to a vigorously stirred solution of alpha D-glucose (20 g, 0.111 mmol) in acetone (400 mL) at 0 ^{o}C. Sulfuric acid (98%, 16 mL) was added dropwise over 15 minutes. After the addition was complete the reaction was stirred vigorously for 5 hours whilst allowing the temperature to rise gradually to room temperature. The reaction mixture was cooled in an ice bath and 50% sodium hydroxide solution (30 mL) was added dropwise. A small amount of solid sodium bicarbonate was added to maintain the solution at near neutral pH. The solution was stirred overnight, filtered and concentrated under reduced pressure to a thick oil. The oil was dissolved in chloroform and washed with water (2 x 100 mL). The aqueous layer was extracted with chloroform (3 x 70 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude solid was recrystallised from cyclohexane to give the title compound **216** (14.13 g, 49%) as white crystals.

mp: 109-110 ^{o}C (Lit., 110 ^{o}C)[59]

$[\alpha]_D^{20}$ = -13.3 (1.35, $CHCl_3$), (Lit., -13.5)[59]

IR: (thin film) 3434, 2987, 2952, 2936, 2807, 2904, 1458, 1423, 1376, 1319, 1287, 1249, 1223 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.28 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 3.97 (dd, 1H, *J* = 5.2, 8.6 Hz, H-6), 4.02 (dd, 1H, *J* = 2.8, 7.7 Hz, H-4), 4.13 (dd, 1H, *J* = 6.3, 8.6 Hz, H-6), 4.28-4.33 (m, 2H, H-3, H-5), 4.49 (d, 1H, *J* = 3.6 Hz, H-2), 5.90 (d, 1H, *J* = 3.6 Hz, H-1).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.23 (CH_3), 26.25 (CH_3), 26.85 (CH_3), 26.91 (CH_3), 67.70 (C-6), 73.34 (C-5), 75.06 (C-3), 81.22 (C-4), 85.16 (C-2), 105.31 (C-1), 109.70 ($Me_2\underline{C}$), 111.89 ($Me_2\underline{C}$).

LRMS (ESI^+): 283.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{12}H_{24}O_6N$ $[M+NH_4]^+$: 278.1598; Found: 278.1606.

3-Vinyl-α-D-1,2,:5,6-di-*O*-isopropylidene glucofuranose **218**

A mixture of $Pd(OAc)_2$ (314 mg, 1.40 mmol) and 4,7-diphenyl-1,10-phenanthroline (641 mg, 1.93 mmol) in BVE (10 mL) was stirred for 5 minutes at room temperature under an argon atmosphere. A solution of compound **216** (12.83 g, 49 mmol) in BVE

(270 mL) was added *via* a cannula to the mixture which was then stirred for 7 days at 75 ^{o}C under an argon atmosphere. The reaction was cooled to room temperature and passed through a column of activated charcoal with petrol/EtOAc (1:1) to remove the catalyst. The crude product was purified by flash chromatography eluting with petrol/EtOAc (15:1) by deactivating the silica gel with Et_3N (2 mL), to give the title compound **218** (12.4 g, 88%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -25.7 (16.9, $CHCl_3$).

IR: (thin film) 2988, 2938, 2895, 1638, 1621, 1457, 1374, 1328 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.30 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 4.02 (dd, 1H, *J* = 5.2, 8.6 Hz, H-6), 4.08 (dd, 1H, *J* = 6.2, 8.6 Hz, H-6)**,** 4.14 (dd, 1H, *J* = 2.3, 6.7 Hz, H-8), 4.18 (dd, 1H, *J* = 2.9, 7.7 Hz, H-4), 4.30 (m 1H, H-5), 4.34 (d, 1H, *J* = 2.9 Hz, H-3), 4.38 (dd, 1H, *J* = 2.3, 14.3 Hz, H-8), 4.58 (d, 1H, *J* = 3.8 Hz, H-2), 5.87 (d, 1H, *J* = 3.8 Hz, H-1), 6.38 (dd, 1H, *J* = 7.6, 14.3 Hz, H-7).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.31 (CH_3), 26.24 (CH_3), 26.75 (CH_3), 26.88 (CH_3), 67.08 (C-6), 72.15 (C-5), 80.38 (C-3, C-4), 82.1 (C-2), 89.43 (C-8), 105.15 (C-1), 109.14 ($Me_2\underline{C}$), 111.91 ($Me_2\underline{C}$), 150.05 (C-7).

LRMS (ESI^+): 287.3 $[M+H]^+$.

HRMS (ESI) calcd for $C_{14}H_{23}O_6$ $[M+H]^+$: 287.1489; Found: 287.1492.

6-Trityl-β-D-glucose-1,2,3,4-tetraacetate **219**

A mixture of D-glucose (15.2g, 84.5 mmol), trityl chloride (24.2 g, 86.8 mmol), and anhydrous pyridine (65 mL) was heated until a solution was obtained. Without cooling, acetic acid (45 mL) was added and the mixture was left to cool and stand for 18 h. The reaction mixture was poured slowly into ice water (1 L), to which acetic acid (500 mL) was added, and the resulting mixture was stirred mechanically for 2 hrs. The precipitate was filtered, and washed with cold water and dried. The solid was suspended in ether (60 mL). The insoluble material was collected, dried and then recrystallised from hot 95% aqueous ethanol to give the title compound **219** (20.9 g, 42%) as fine needles.

mp: 166-167 °C (Lit., 166–166.5 °C)[66]

$[\alpha]_D^{20}$ = +44.4 (*c* 4.74, $CHCl_3$), (Lit., +44.8)[66]

IR: (KBr): 3024, 2943, 1757, 1432, 1223, 1078, 1039, 901, 757 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.72 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 3.05 (dd, 1H, *J* = 4.1, 10.5 Hz, H-6), 3.33 (dd, *J* = 2.1, 10.5 Hz, H-6), 3.68 (m, 1H, H-5), 5.17 (m, 2H, H-2, H-3), 5.26 (m, 1H, H-4), 5.71 (dd, 1H, *J* = 3.2, 1.5 Hz, H-1), 7.22 (m, 3H, Ar-H), 7.26 (m, 6H, Ar-H), 7.41 (d, 6H, *J* = 11.5 Hz, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.54 (CH_3), 20.71 (CH_3), 20.75 (CH_3), 20.98 (CH_3), 61.71 (C-6), 68.37 (C-4), 70.61 (C-3), 73.23 (C-2), 74.13 (C-5), 86.72 (O<u>C</u>Ph_3), 92.01 (C-1), 127.11 (Ar-CH), 127.88 (Ar-CH), 128.80 (Ar-CH), 143.56 (Ar-C), 169.04 (C=O), 169.09 (C=O), 169.46 (C=O), 170.37 (C=O).

LRMS (ESI^+): 613.4 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{33}H_{38}O_{10}N$ $[M+NH_4]^+$: 608.2490 ; Found: 608.2490.

β-D-Glucose-1,2,3,4-tetraacetate **220**

Compound **219** (20.3 g, 34.4 mmol) and acetic acid (100 mL) were heated until a solution was obtained. The solution was cooled to 10 ^oC, and a solution of hydrogen bromide in acetic acid (8.2 mL) was added, and the mixture was shaken for 45 seconds. The mixture was immediately filtered to remove the trityl bromide formed, and the filtrate is poured immediately into cold water (500 mL). The product was extracted with chloroform (200 mL) and washed with ice water (4 x 50 mL) and dried over anhydrous sodium sulphate. The chloroform was evaporated under reduced pressure. The resulting syrup was covered with anhydrous ether to induce crystallisation. The crude product was removed by filteration and dissolved in the minimum amount of chloroform, followed by slow, careful addition of diethyl ether until crystallisation began, to give the title compound **220** (6.28 g, 53%) as a white crystalline solid.

mp: 127-128 °C, (Lit., 128–129 °C)[66]

$[\alpha]_D^{20}$ = +10.8 (*c* 4.13, $CHCl_3$), (Lit., +12.1)[66]

IR: (KBr) 3475, 3060, 2938, 2871, 1751, 1492, 1449, 1366, 1224, 1072, 980 cm^{-1}

^1H NMR ($CDCl_3$, 600 MHz): δ 2.01 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.28 (br s, 1H, OH), 3.56 (dd, 1H, *J* = 4.0, 12.5 Hz, H-6), 3.65 (ddd, 1H, *J* = 1.7, 4.0, 6.0 Hz, H-5), 3.77 (dd, 1H, *J* = 6.0, 12.5 Hz, H-6), 5.06-5.12 (m, 2H, H-2 and H-4), 5.28-5.31 (m, 1H, H-3), 5.71 (d, 1H, *J* = 8.2, H-1).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.72 (CH_3), 20.75 (CH_3), 20.78 (CH_3), 20.96 (CH_3), 60.93 (C-6), 68.28 (C-4), 70.50 (C-3), 72.71 (C-2), 75.01 (C-5), 91.83 (C-1), 169.24 (C=O), 169.44 (C=O), 170.26 (C=O), 170.46 (C=O).

LRMS (ESI^+): 371.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{14}H_{20}O_{10}Na$ $[M+Na]^+$: 371.0949; Found: 371.0949.

6-Vinyloxy-β-D-glucose-1,2,3,4-tetraacetate ***221***

A mixture of $Pd(OAc)_2$ (88 mg, 0.39 mmol) and 4,7-diphenyl-1,10-phenanthroline (180 mg, 0.54 mmol) in BVE (10 mL) was stirred for 5 minutes at room temperature. A solution of compound **220** (3.60 g, 10.3 mmol) in BVE (65 mL) was added *via* cannula to this mixture, which was then stirred for 7 days at 75 ^{o}C under an argon atmosphere. The reaction mixture was cooled to room temperature and was passed through column of activated charcoal eluting with petrol/EtOAc (1:1) to remove the catalyst. The product was concentrated under reduced pressure and recrystallised from petrol to afford the *title compound* **221** (1.74g, 45%) as a white solid.

mp: 101-103 °C

$[\alpha]_D^{20}$ = 12.7 (*c* 6.02, $CHCl_3$).

IR: (KBr): 2958, 2954, 1761, 1625, 1372, 1223, 1079, 1039, 901 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.01 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.75 (dd, 1H, *J* = 4.6, 11.2 Hz, H-6), 3.81 (dd, 1H, *J* = 2.6, 11.2 Hz, H-6), 3.86-3.89 (m, 1H, H-5), 4.03 (dd, 1H, *J* = 2.4, 6.7 Hz, H-8 *cis*), 4.15 (dd, 1H, *J* =

2.4, 14.3 Hz, H-8 *trans*), 5.12-5.19 (m, 2H, H-2, H-4), 5.26 (t, 1H, *J* = 9.5 Hz, H-3), 5.73 (d, 1H, *J* = 8.3, H-1), 6.44 (dd, 1H, *J* = 6.7, 14.3 Hz, H-7)**.**

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.66 (CH_3), 20.71 (CH_3), 20.92 (CH_3), 31.04 (CH_3), 65.92 (C-6), 68.52 (C-4), 70.35 (C-2), 72.93 (C-3), 73.07 (C-5), 87.36 (C-8), 91.78 (C-1), 151.38 (C-7), 169.04 (C=O), 169.09 (C=O), 169.46 (C=O), 170.37 (C=O).

LRMS (ESI^+): 392.1 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{16}H_{26}O_{10}N_1$ $[M+NH_4]^+$: 392.1551; Found: 392.1558.

1,2,3,4,6-Penta-*O*-benzoyl-α-D-mannopyranose **223**

D-Mannose (2.0 g, 11.1 mmol) and a catalytic DMAP were dissolved in pyridine (12 mL) and cooled to 0 ^oC. Benzoyl chloride (9.6 mL, 82.5 mmol) was added dropwise and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in DCM (200 mL) and H_2O (40 mL) was added carefully with cooling and vigorous stirring to decompose excess benzoyl chloride. The product was extracted with DCM, the organic phase was washed with brine (40 mL), sat. $NaHCO_3$ (2 × 40 mL), and brine (40 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced preaure. The crude product was dissolved in a mixture of acetone–methanol (1:1, *ca.* 20 mL), and left overnight. The solution was then diluted with methanol (10 mL), left for 2 h, further diluted with

methanol (20 mL) and left for two days at 4 ^{o}C. The precipitated crystals were collected by filtration, washed with a small amount of methanol and dried in air to give the title compound **223** (5.56 g, 72%) as a white solid.

mp: 151–152 ^{o}C (Lit., 152-153 ^{o}C)[159]

$[\alpha]_D^{20}$ = -22.1 (*c* 2.09, $CHCl_3$), (Lit., -18.6)[159]

IR: (KBr): 3064, 3034, 2973, 2913, 2890, 1725, 1602, 1453, 1266, 1178, 1086, 1026, 995 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.49 (dd, 1H, *J* = 3.6, 12.2 Hz, H-6), 4.56 (dt, 1H, *J* = 10.1, 2.5 Hz, H-5), 4.69 (dd, 1H, *J* = 2.4, 12.2 Hz, H-6), 5.90-5.91 (m, 1H, H-2), 6.06 (dd, 1H, *J* = 3.3, 10.1 Hz, H-3), 6.28 (t, 1H, *J* = 10.1 Hz, H-4), 6.62 (s, 1H, H-1), 7.27-7.69 (m, 15H, Ar-H), 7.84-8.20 (m, 10H, Ar-H),

^{13}C NMR ($CDCl_3$, 150.9 MHz): 62.45 (C-6), 66.26 (C-4), 69.54 (C-2), 70.11 (C-3), 71.30 (C-5), 91.48 (C-1), 128.56-129.13 (Ar-C, Ar-CH), 128.97 (Ar-CH), 129.92 (Ar-CH), 129.97 (Ar-CH), 130.11 (Ar-CH), 130.31 (Ar-CH), 133.22 (Ar-CH), 133.56 (Ar-CH), 133.71 (Ar-CH), 133.83 (Ar-CH), 134.24 (Ar-CH), 163.96 (C=O), 165.27 (C=O), 165.41 (C=O), 165.85 (C=O), 166.18 (C=O).

LRMS (ESI^+): 723 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{41}H_{36}O_{11}N$ $[M+NH_4]^+$: 718.2283; Found: 718.2286.

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranose **224**

Ethanolamine (1.14 mL, 15.76 mmol) was added dropwise to a solution of compound **223** (12.1 g, 17.30 mmol) in dry THF (100 mL) and. The solution was stirred overnight, during which time a white precipitate formed. The precipitate was removed by filteration, and the filtrate was stripped of solvent under reduced pressure. The crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (3:1) to give the title compound **224** (5.1 g, 50%) as a white foam.

$[\alpha]_D^{20}$ = -83.5 (*c* 0.4, $CHCl_3$), (Lit., -81.0)[160]

IR: (KBr): 3501, 3067, 2957, 1726, 1602, 1452, 1269, 1179, 1107, 1069, 1025, 969 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.58 (br s, 1H, OH), 4.45 (dd, 1H, *J* = 3.6, 12.2 Hz, H-6), 4.68 (dt, 1H, *J* = 10.0, 2.9 Hz, H-5), 4.77 (dd, 1H, *J* = 2.4, 12.2 Hz, H-6), 5.54 (s, 1H, H-1), 5.74-5.75 (m, 1H, H-2), 6.01 (dd, 1H, *J* = 3.3, 10.3 Hz, H-3), 6.18 (t, 1H, *J* = 10.1 Hz, H-4), 7.27-7.59 (m, 12H, Ar-H), 7.84-8.13 (m, 8H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): 62.83 (C-6), 66.96 (C-4), 69.04 (C-5), 69.86 (C-3), 70.93 (C-2), 91.48 (C-1), 128.44 (Ar-CH), 128.59 (2 x Ar-CH), 128.70 (Ar-CH), 128.80 (Ar-C), 129.12 (Ar-C), 129.20 (Ar-C), 129.40 (Ar-C), 129.89 (Ar-CH), 129.95 (2 x Ar-

CH), 129.97 (Ar-CH), 133.23 (Ar-CH), 133.32 (Ar-CH), 133.57 (Ar-CH), 133.59 (Ar-CH), 165.61 (2 x C=O), 165.69 (C=O), 166.46 (C=O).

LRMS (ESI^+): 619 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{34}H_{32}O_{10}N$ $[M+NH_4]^+$: 614.2021; Found: 614.2018.

1-Vinyl-2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranose **225**

A mixture of $Pd(OAc)_2$ (194 mg, 0.864 mmol) and 4,7-diphenyl-1,10-phenanthroline (323 mg, 97.1 mmol) in BVE (10 mL) was stirred for 5 minutes at room temperature. Compound **224** (3.60 g, 10.3 mmol) was dissolved in BVE (170 mL) and injected into the reaction mixture which was then stirred for 7 days at 75 ^oC in an argon atmosphere. The reaction mixture was cooled to room temperature and was passed through a column of activated charcoal eluting with petrol/EtOAc (1:1) to remove the catalyst. The product was concentrated under reduced pressure and purified by flash chromatography eluting with petroleum ether/EtOAc (3:1) to give the title compound **225** (3.6 g, 46%) as a yellow oil.

$[\alpha]_D^{20}$ = -37.1 (*c* 7.00, $CHCl_3$).

IR: (thin film) 3066, 2959, 2935, 2872, 1730, 1646, 1602, 1268, 1103, 1070, 1027, 978 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.36 (dd, 1H, *J* = 2.1, 6.5 Hz, H-8), 4.43-4.4.48 (m, 2H, H-5, H-6), 4.70 (d, 1H, *J* = 11.5 Hz, H-6), 4.79 (dd, 1H, *J* = 2.1, 14.1 Hz, H-8), 5.45 (s, 1H, H-1), 5.78 (dd, 1H, *J* = 1.9, 3.4 Hz, H-2), 5.97 (dd, 1H, *J* = 3.4, 10.1 Hz, H-3), 6.16 (t, 1H, *J* = 10.1 Hz, H-4), 6.49 (dd, 1H, *J* = 6.5, 14.1 Hz, H-7), 7.27-7.62 (m, 12H, Ar-H), 7.84-8.12 (m, 8H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): 62.66 (C-6), 66.61 (C-4), 69.53 (C-5), 69.89 (C-3), 69.93 (C-2), 93.90 (C-7), 96.39 (C-1), 128.46 (Ar-CH), 128.57 (Ar-CH), 128.60 (Ar-CH), 128.70 (Ar-C), 128.76 (Ar-CH), 128.99 (Ar-C), 129.08 (Ar-C), 129.24 (Ar-C), 129.89 (2 x Ar-CH), 129.96 (Ar-CH), 130.01 (Ar-CH), 133.21 (Ar-CH), 133.39 (Ar-CH), 133.64 (Ar-CH), 133.72 (Ar-CH), 147.58 (C-8), 165.44 (C=O), 165.54 (C=O), 166.60 (C=O), 166.26 (C=O).

LRMS (ESI^+): 645 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{36}H_{34}O_{10}N$ $[M+NH_4]^+$: 640.2177; Found: 640.2173.

Ethyl coumalate **227**

4-Dimethylaminopyridine (1.17 g, 9.58 mmol) was added to a stirred solution of coumalic acid (6.77 g, 48.3 mmol) in DCM (2.50 mL) and stirred for 15 min at room temperature. Ethanol (14 mL) and dicyclohexyl carbodiimide (9.9 g, 48 mmol) were added and the reaction mixture stirred for 18 hours. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (200 mL) and filtered through a bed of celite™. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (2:1) to give the title compound (6.1 g, 75%) as a yellow solid.

mp: 42-43 ^{o}C (Lit, 43 ^{o}C)[69]

IR: (KBr) 3088, 2986, 2940, 1757, 1722, 1555, 1469, 1432, 1370, 1296, 1238, 1086, 1019, 948 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.35 (t, 3H, *J* = 7.2 Hz, H-9), 4.33 (q, 2H, *J* = 7.2 Hz, H-8), 6.33 (dt, 1H, *J* = 9.8, 1.0 Hz, H-3), 7.79 (dd, 1H, *J* = 2.6, 9.8 Hz, H-4), 8.29-8.30 (dd, *J* = 1.0, 2.6 Hz, H-6).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.33 (C-9), 61.76 (C-8), 112. 31 (C-5), 115.34 (C-3), 141.87 (C-4), 158.14 (C-6), 160.06 (C=O), 163.07 (C=O).

LRMS (ESI^+): 169.1 $[M+H]^+$.

HRMS (ESI) calcd for $C_8H_9O_4$ $[M+H]^+$: 169.9495; Found: 169.0495.

3-Bromo-2(*H*)-pyran-2-one **228** & 5-bromo-2(*H*)-pyran-2-one **229**

Triethylamine (4.3 mL) was added dropwise to a stirred solution of 3,5-dibromo-5,6-dihydro-2H-pyran-2-one[161] (6.27 g, 24.51 mmol) in DCM (110 mL) and the reaction mixture stirred for 24 hours. The reaction mixture was washed with water (3 x 100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (10:1) to give the title compound **229** (300 mg, 7%) as a light brown solid and the title compound **228** (1.39 g, 33%) as a tan solid.

3-Bromo-2(*H*)-pyran-2-one **228**

IR: (KBr) 3098, 1730, 1622, 1530, 1322, 1236, 1092, 973 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 6.16 (dd, 1H, *J* = 5.0, 7.0 Hz, H-5), 7.33 (dd, 1H, *J* = 1.7, 5.0 Hz, H-6), 7.70 (dd, 1.7, 7.0 Hz, H-4).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 106.67 (C-5), 112.89 (C-3), 144.19 (C-4), 151.07 (C-6), 158.32 (C=O).

LRMS (ESI^+): 175.0 $[M+H]^+$.

HRMS (ESI) calcd for $C_5H_4O_2{}^{79}Br$ $[M+H]^+$: 174.9386; Found: 174.9386.

5-Bromo-2(*H*)-pyran-2-one **229**

IR: (KBr) 3073, 1744, 1606, 1531, 1322, 1220, 1156, 1126, 1040, 938, 830 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 6.29 (d, 1H, *J* = 10.0 Hz, H-3), 7.33 (dd, 1H, *J* = 2.7, 10.0 Hz, H-4), 7.58 (d, 2.7 Hz, H-6).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 100.93 (C-5), 117.72 (C-3), 146.13 (C-4), 149.81 (C-6), 159.61 (C=O).

LRMS (ESI^+): 175.0 $[M+H]^+$.

HRMS (ESI) calcd for $C_5H_3O_2{}^{79}Br$ $[M+H]^+$: 173.9311; Found: 173.9313.

Cycloaddition between **218** & **226**

A solution of compound **218** (8.39 g, 29.32 mmol, 2 eq.) and compound **226** (2.26 g, 14.66 mmol, 1 eq.) in DCM (2 mL) was heated for 7 days at 65 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (15:1, then 8:1, then 5:1) to give 5-*endo* cycloadduct **246a** (2.97 g, 43%) as a pale yellow oil, 5-*endo* cycloadduct **246b** (1.56 g, 24%) as a white crystalline solid, and a mixture of 5-*exo* cycloadducts **246c/d** (4.6:1, 204 mg, 3%) as a yellow oil.

5-Endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester **246a**

$[\alpha]_D^{20}$ = -14.1 (*c* 8.00, $CHCl_3$).

IR: (thin film) 2988, 2939, 2897, 1766, 1619, 1439, 1373, 1248, 1284, 1218, 1119, 1077 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.92 (dt, 1H, *J* = 13.8, 1.7 Hz, H-6), 2.59 (ddd, 1H, *J* = 3.6, 7.2, 13.8 Hz, H-6), 3.87 (m, 1H, H-6'), 3.91 (m, 1H, H-4') 3.93 (s, 3H, OCH_3), 3.99-4.00 (m, 1H, H-3'), 4.01-4.04 (m, 2H, H-5', H-6') 4.59 (m, 2H, H-2', H-5), 5.27 (ddd, 1H, *J* = 1.7, 3.6, 5.2 Hz, H-1), 5.73 (d, 1H, *J* = 3.8 Hz, H-1'), 6.60 (dd, 1H, *J* = 5.2, 7.7 Hz, H-7), 6.71 (d, 1H, *J* = 7.7 Hz, H-8).

^{13}C NMR ($CDCl_3$): δ 25.48 (CH_3), 26.27 (CH_3), 26.88 (CH_3), 26.91 (CH_3), 34.68 (C-6), 53.22 (OCH_3), 61.48 (C-4), 67.67 (C-6'), 71.91 (C-5'), 72.20 (C-5), 74.24 (C-1), 80.66 (C-4'), 80.92 (C-3'), 81.93 (C-2'), 105.51 (C-1'), 109.69 ($Me_2\underline{C}$), 112.17 ($Me_2\underline{C}$), 129.60 (C-8), 130.76 (C-7), 167.45 (C=O), 168.35 (C=O).

LRMS (ESI^+): 463.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{21}H_{29}O_{10}$ $[M+H]^+$: 441.1755; Found: 441.1757.

5-Endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester ***246b***

mp: 147-149 ^{o}C

$[\alpha]_D^{20}$ = -13.5 (*c* 11.3, $CHCl_3$).

IR: (KBr) 2988, 2925, 2855, 1760, 1738, 1615, 1459, 1440, 1377, 1356 cm^{-1}.

1H NMR ($CDCl_3$): δ 1.30 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.91 (dt, 1H, *J* = 14.1, 1.7 Hz, H-6), 2.76 (ddd, 1H, *J* = 3.6, 7.9, 13.9 Hz, H-6), 3.87 (dd, 1H, *J* = 5.7, 8.4 Hz, H-6'), 3.93 (s, 3H, OCH_3), 4.00 (m, 2H, H-4', H-6'), 4.07 (dd, 1H, *J* = 5.8, 13.8 Hz, H-5'), 4.18 (d, 1H, *J* = 2.9 Hz, H-3'), 4.38 (d, 1H, *J* = 3.6 Hz, H-2'), 4.65 (d, 1H, *J* = 7.4 Hz, H-5), 5.25 (ddd, 1H, *J* = 1.7, 3.4, 6.7 Hz, H-1), 5.76 (d, 1H, *J* = 3.6 Hz, H-1'), 6.62 (dd, 1H, *J* = 2.2, 7.7 Hz, H-7), 6.75 (d, 1H, *J* = 1.2, 7.7 Hz, H-8).

13C NMR ($CDCl_3$): δ 25.31 (CH3), 26.34 (CH_3), 26.88 (CH_3), 26.90 (CH_3), 37.32 (C-6), 53.14 (OCH3), 61.67 (C-4), 67.37 (C-6'), 71.93 (C-5'), 72.07 (C-5), 73.91 (C-1), 81.13 (C-4'), 82.70 (C-3') 82.89 (C-2'), 105.03 (C-1'), 109.20 ($Me_2\underline{C}$), 112. 28 ($Me_2\underline{C}$), 129.96 (C-8), 130.73 (C-7), 167.20 (C=O), 168.45 (C=O).

LRMS (ESI^+): 463.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{21}H_{32}NO_{10}$ $[M+NH_4]^+$: 458.2021 ; Found: 458.2020.

5-Exo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester **246c/d**

IR: (thin film) 2989, 1743, 1439, 1626, 1439, 1369, 1218, 1077, 1019 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 2.1 (dd, 1H, *J* = 8.4, 13.6 Hz, H-6), 2.32 (ddd, 1H, *J* = 2.2, 4.0, 13.6 Hz, H-6), 3.90-3.91 (m, 1H, H-6'), 3.92 (s, 3H, OCH_3), 4.03 (dd, 1H, *J* = 3.1, 8.6 Hz, H-4'), 4.06-4.08 (m, 2H, H-3', H-6'), 4.11-4.4.15 (m, 1H, H-5'), 4.30 (dd, 1H, *J* = 2.1, 8.4 Hz, H-5), 4.78 (d, 1H, *J* = 3.6 Hz, H-2'), 5.23-5.24 (m, 1H, H-1), 5.84 (d, 1H, *J* = 3.6 Hz, H-1'), 6.36 (dd, 1H, *J* = 2.2, 7.7 Hz, H-8), 6.75 (d, 1H, *J* = 1.2, 7.7 Hz, H-7).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.46 (CH_3), 26. 46 (CH_3), 27.03 (CH_3), 27.07 (CH_3), 34.64 (C-6), 53.23 (OCH_3), 60.12 (C-4), 67.87 (C-6'), 72.46(C-5'), 73.59 (C-5), 73.89 (C-1), 81.26 (C-4'), 82.17 (C-3') 82.42 (C-2'), 105.68 (C-1'), 109.13 ($Me_2\underline{C}$), 111.96 ($Me_2\underline{C}$), 130.5 (C-8), 134.70 (C-7), 166.66 (C=O), 168.58 (C=O).

LRMS (ESI^+): 463 $[M+Na]^+$.

HRMS(EI) calcd for $C_{21}H_{32}O_{10}N$ $[M+NH_4]^+$: 458.2021; Found: 458.2024.

Cycloaddition between **218** & **227**

A solution of compound **218** (1.98 g, 6.92 mmol, 2 eq.) and ethyl coumalate **227** (581 mg, 3.46 mmol, 1 eq.) in DCM (2 mL) was heated for 6 days at 100 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (8:1 and 6:1) to give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **247a/b** (1.22 g, 78%), as a pale yellow oil, a mixture of 5-*exo*-1/5-*exo*-2 cycloadducts **247c/d** (162 mg, 10%) as a pale yellow oil and a mixture of diastereomers **272a/b** (159 mg, 11%) as a pale yellow oil.

5-Endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester **247a**

IR: (thin film) 2987, 2938, 1767, 1717, 1637, 1456, 1379, 1300, 1258, 1216, 1080, 960 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz**):** δ 1.31 (br s, 3H, OCH_2CH_3), 1.33 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.82 (dt, 1H, *J* = 14.3, 1.4 Hz, H-6),

2.57 (ddd, 1H, *J* = 3.8, 7.6, 14.3 Hz, H-6), 3.90 (dd, 1H *J* = 5.0, 8.4 Hz, H-6'), 3.92 (d, 1H, *J* = 2.9 Hz, H-4'), 3.96-3.98 (m, 1H, H-6'), 3.99-4.01 (m, 1H, H-3'), 4.02-4.06 (m, 1H, H-4), 4.22-4.26 (m, 3H, O*CH*$_2$CH$_3$, H-5'), 4.32-4.34 (m, 1H, H-5), 4.43 (d, 1H, *J* = 3.6 Hz, H-2'), 5.71-5.72 (m, H-1), 5.83 (d, 1H, *J* = 3.6 Hz, H-1'), 7.23 (dd, 1H, *J* = 1.4, 6.2 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.29 (OCH$_2$*CH$_3$*), 25.19 (CH$_3$), 26. 31 (CH$_3$), 26.84 (CH$_3$), 26.98 (CH$_3$), 34.10 (C-6), 48.55 (C-4), 61.48 (O*CH$_2$*CH$_3$), 67.84 (C-6'), 71.36 (C-5'), 72.08 (C-5), 73.38 (C-1), 80.69 (C-4'), 81.18 (C-3'), 83.11 (C-2'), 105.50 (C-1'), 109.31 (Me$_2$C), 112.29 (Me$_2$C), 136.44 (C-8), 137.92 (C-7), 162.01 (C=O), 170.23 (C=O).

5-Endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester **247b**

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.30 (br s, 3H, OCH$_2$C*H$_3$*), 1.31 (s, 3H, CH$_3$), 1.39 (s, 3H, CH$_3$), 1.44 (s, 3H, CH$_3$), 1.48 (s, 3H, CH$_3$), 1.59 (dt, 1H, *J* = 1.4, 14.3 Hz, H-6), 2.68 (ddd, 1H, *J* = 3.8, 7.9, 14.3 Hz, H-6), 3.95 (dd, 1H, *J* = 4.6, 8.3 Hz, H-6'), 3.97-4.00 (m, 2H, H-3', H-4'), 4.08 (dd, 1H, *J* = 6.2, 8.3 Hz, H-6'), 4.16 (dd, 1H, *J* = 3.3, 6.2 Hz, H-4), 4.23-4.26 (m, 3H, O*CH$_2$*CH$_3$, H-5'), 4.32-4.33 (m, 1H, H-5), 4.42 (d, 1H, *J* = 3.8 Hz, H-2'), 5.68-5.69 (m, 1H, H-1), 5.80 (d, 1H, *J* = 3.8 Hz, H-1'), 7.26-7.27 (m, 1H, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.28 (OCH$_2$*CH$_3$*), 25.34 (CH$_3$), 26.27 (CH$_3$), 26.87 (CH$_3$), 26.90 (CH$_3$), 35.37 (C-6), 46.91 (C-4), 61.44 (O*CH$_2$*CH$_3$), 67.80 (C-6'), 70.87 (C-5'), 71.74 (C-5), 73.27 (C-1), 80.66 (C-4'), 80.96 (C-3'), 83.02 (C-2'), 105.42 (C-1'),

109.55 ($Me_2\underline{C}$), 112.36 ($Me_2\underline{C}$), 135.92 (C-8), 138.89 (C-7), 162.20 (C=O), 170.40 (C=O).

LRMS (ESI^+): 477.0 (MNa^+).

HRMS (ESI) calcd for $C_{22}H_{34}O_{10}N$ $[M+NH_4]^+$: 472.2177; Found: 472.2173.

5-Exo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester **247c/d**

IR: (thin film) 2988, 2939, 1769, 1717, 1637, 1456, 1374, 1252, 1087, 1020, 957 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.30 (overlapping s, 6H, $OCH_2C\mathit{H_3}$, CH_3), 1.31 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.13-2.15 (m, 2H, H-6, H-6), 3.93-3.95 (m, 2H, H-4, H-6'), 3.98 (d, 1H, *J* = 2.1 Hz, H-3'), 4.02 (dd, 1H, *J* = 2.9, 8.6 Hz, H-4'), 4.08-4.15 (m, 3H, H-5, H-5', H-6'), 4.25 (q, 2H, *J* = 7.2 Hz, $O\mathit{CH_2}CH_3$), 4.62 (d, 1H, *J* = 3.6 Hz, H-2'), 5.63-5.64 (m, H-1), 5.83 (d, 1H, *J* = 3.6 Hz, H-1'), 7.21-7.22 (d, 1H, *J* = 2.4 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.28 ($OCH_2\mathit{CH_3}$), 25.46 (CH_3), 26. 36 (CH_3), 26.97 (CH_3), 27.01 (CH_3), 33.61 (C-6), 48.27 (C-4), 61.65 ($O\mathit{CH_2}CH_3$), 67.88 (C-6'), 72.52 (C-5'), 73.53 (C-1), 74.61 (C-5), 81.30 (C-4'), 81.97 (C-3'), 83.69 (C-2'), 105.62 (C-1'), 109.27 ($Me_2\underline{C}$), 112.15 ($Me_2\underline{C}$), 137.32 (C-8), 138.86 (C-7), 161.98 (C=O), 169.92 (C=O).

LRMS (ESI^+): 477.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{22}H_{34}O_{10}N$ $[M+NH_4]^+$: 472.2177; Found: 472.2174.

2-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-cyclohexa-3,5-dienecarboxylic acid ethyl ester ***272a/b***

IR: (thin film) 2986, 2938, 1721, 1454, 1357, 1250, 1217, 1163, 1075, 1022, 960 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.28 (br s, 3H, $OCH_2C\mathit{H_3}$), 1.32 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.50 (ddd, 1H, *J* = 3.1, 7.6, 19.6 Hz, H-1), 2.79 (dt, 1H, *J* = 19.6, 5.0 Hz, H-1), 3.93 (dd, 1H, *J* = 6.0, 8.6 Hz, H-6'), 4.00 (dd, 1H, *J* = 2.9, 9.5 Hz, H-4'), 4.03-4.08 (m, 2H, H-3', H-6'), 4.17-4.19 (m, 1H, H-2), 4.20-4.21 (m, 1H, H-5'), 4.22-4.23 (m, 2H, $O\mathit{CH_2}CH_3$), 4.45 (d, 1H, *J* = 3.6 Hz, H-2'), 5.82

(d, 1H, *J* = 3.6 Hz, H-1'), 6.04 (dd, 1H, *J* = 4.6, 9.8 Hz, H-3), 6.62 (d, 1H, *J* = 9.8 Hz, H-4), 6.97-7.00 (m, 1H, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.35 ($OCH_2\mathit{CH_3}$), 25.51 (CH_3), 26. 37 (CH_3), 26.94 (2 x CH_3), 29.94 (C-1), 60.74 ($O\mathit{CH_2}CH_3$), 67.69 (C-6'), 69.89 (C-5'), 72.31 (C-2), 80.05 (C-4'), 81.42 (C-3'), 84.10 (C-2'), 105.44 (C-1'), 109.08 ($Me_2\underline{C}$), 111.93 ($Me_2\underline{C}$), 124.86 (C-4), 125.08 (C-3), 136.37 (C-5a), 159.70 (C-5), 165.33 (C=O).

LRMS (ESI^+): 428.2 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{21}H_{34}O_8N$ $[M+NH_4]^+$: 428.2279; Found: 428.2274.

Cycloaddition between **218** & **228**

A solution of compound **218** (2.13 g, 7.44 mmol, 20 eq.) and compound **228** (65.1 mg, 0.372 mmol, 1 eq) in DCM (1 mL) and a few drops of Et_3N was heated for 6 days at 100 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting

with petroleum ether/EtOAc (10:1) to give 5-*endo*-cycloadduct **248a** (65 mg, 38%), as a yellow oil and 5-*endo*-cycloadduct **248b** (52 mg, 30%) as a yellow oil.

IR: (thin film) 2989, 2938, 1763, 1620, 1439, 1374, 1284, 1217, 1077, 1023, 959 cm^{-1}.

4-Bromo-5-endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***248a***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.91 (dt, 1H, *J* = 13.9, 1.7 Hz, H-6), 2.58 (ddd, 1H, *J* = 3.6, 7.4, 13.9 Hz, H-6), 3.87-3.88 (m, 1H, H-6'), 3.91 (d, 1H, *J* = 2.8 Hz, H-4'), 3.98-4.04 (m, 3H, H-3', H-5', H-6'), 4.57-4.58 (m, 1H, H-5), 4.58 (d, 1H, *J* = 3.6 Hz, H-2'), 5.29-5.31 (m, 1H, H-1), 5.74 (d, 1H, *J* = 3.6 Hz, H-1'), 6.60 (dd, 1H, *J* = 5.0, 7.7 Hz, H-7), 6.71 (d, 1H, *J* = 7.7 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.55 (CH_3), 26.32 (CH_3), 26.94 (CH_3), 26.96 (CH_3), 34.74 (C-6), 61.54 (C-4), 67.67 (C-6'), 71.91 (C-5'), 72.20 (C-5), 74.24 (C-1), 80.74 (C-4'), 80.98 (C-3'), 82.00 (C-2'), 105.57 (C-1'), 1109.17 ($Me_2\underline{C}$), 112.31 ($Me_2\underline{C}$), 129.67 (C-8), 130.80 (C-7), 167.52 (C=O).

LRMS (ESI^+): 463.0 $[M+H]^+$.

HRMS (ESI) calcd for $C_{19}H_{29}O_8N^{79}Br$ $[M+NH_4]^+$: 478.1071; Found: 478.1075.

4-Bromo-5-endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***248b***

IR: (thin film) 2989, 2958, 2932, 1767, 1634, 1457, 1374, 1165, 1082, 1029 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.29 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.81 (dt, 1H, *J* = 13.8, 1.7 Hz, H-6), 2.76 (ddd, 1H, *J* = 2.4, 7.7, 13.8 Hz, H-6), 3.93 (dd, 1H, *J* = 6.2, 8.4 Hz, H-6'), 4.04 (dd, 1H, *J* = 6.5, 8.4 Hz, H-6'), 4.10 (m, 1H, H-5'), 4.22 (dd, 1H, *J* = 2.6, 6.9 Hz, H-4'), 4.24 (dt, 1H, *J* = 1.2, 7.7 Hz, H-5), 4.28 (d, 1H, *J* = 3.1 Hz, H-3'), 4.36 (d, 1H, *J* = 3.6 Hz, H-2'), 5.24 (ddd, 1H, *J* = 1.7, 3.6, 6.9 Hz, H-1), 5.86 (d, 1H, *J* = 3.8 Hz, H-1'), 6.44 (m, 1H, H-7), 6.49 (m, 1H, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.48 (CH_3), 26.27 (CH_3), 26.88 (CH_3), 26.91 (CH_3), 34.68 (C-6), 61.48 (C-4), 67.74 (C-6'), 71.98 (C-5'), 72.26 (C-5), 74.30 (C-1), 80.66 (C-4'), 80.92 (C-3'), 81.93 (C-2'), 105.51 (C-1'), 109.69 ($Me_2\underline{C}$), 112.17 ($Me_2\underline{C}$), 129.60 (C-8), 130.76 (C-7), 167.45 (C=O).

LRMS (ESI^+): 463.0 $[M+H]^+$.

HRMS (ESI) calcd for $C_{19}H_{29}O_8N^{79}Br$ $[M+NH_4]^+$: 478.1071; Found: 478.1070.

Cycloaddition between **218** & **229**

A solution of compound **218** (1.10 g, 3.84 mmol, 10 eq.) and compound **229** (67 mg, 0.384 mmol, 1 eq.) in DCM (1 mL) and a few drops of Et_3N was heated for 6 days at 100 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (8:1) to give a mixture of 5-*endo*-1/5-*endo*-2/5-*exo*-1 cycloadducts **249a/b/c** (157 mg, 89%) as a yellow oil.

7-Bromo-5-endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***249a***

IR: (thin film) 2988, 2937, 2899, 1768, 1618, 1456, 1374, 1215, 1163, 1082, 1022, 913 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.29 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.48, (s, 3H, CH_3), 1.74 (d, 1H, *J* = 14.3 Hz, H-6), 2.61 (ddd, 1H, *J* = 3.8, 7.9, 14.3 Hz, H-6), 3.91 (d, 1H, *J* = 2.9 Hz, H-4'), 3.92 (dd, 1H, *J* = 2.9 Hz, 8.9 Hz, H-6'), 3.95-3.98 (m, 3H, H-4, H-3', H-6'), 4.06-4.10 (m, 1H, H-5'), 4.15-4.18 (m, 1H, H-5), 4.44 (d, 1H,

J = 3.6 Hz, H-2'), 5.09-5.10 (m, 1H, H-1), 5.82 (d, 1H, *J* = 3.6 Hz, H-1'), 6.49 (dd, 1H, *J* = 2.2, 6.4 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.51 (CH_3), 26.29 (CH_3), 26.83 (CH_3), 27.01 (CH_3), 35.53 (C-6), 47.87 (C-4), 67.73 (C-6'), 70.43 (C-5'), 71.80 (C-5), 80.37 (C-4'), 80.51 (C-1), 80.89 (C-3'), 83.00 (C-2'), 105.41 (C-1'), 109.48 ($Me_2\underline{C}$), 112.31 ($Me_2\underline{C}$), 119.92 (C-7), 128.43 (C-8), 169.94 (C=O).

7-Bromo-5-endo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***249b***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.30 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.48, (s, 3H, CH_3), 1.96 (d, 1H, *J* = 14.3 Hz, H-6), 2.51 (ddd, 1H, *J* = 3.8, 7.6, 14.3 Hz, H-6), 3.85 (dd, 1H, *J* = 3.3, 6.4 Hz, H-4), 3.95-3.98 (m, 3H, H-3', H-4', H-6'), 4.06-4.10 (m, 2H, H-5', H-6'), 4.22-4.24 (m, 1H, H-5), 4.41 (d, 1H, *J* = 3.8 Hz, H-2'), 5.11-5.12 (m, 1H, H-1), 5.84 (d, 1H, *J* = 3.8 Hz, H-1'), 6.47 (dd, 1H, *J* = 2.2, 6.4 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.46 (CH_3), 26.29 (CH_3), 26.86 (CH_3), 26.93 (CH_3), 34.40 (C-6), 49.47 (C-4), 67.73 (C-6'), 70.95 (C-5'), 72.01 (C-5), 80.51 (C-1), 80.58 (C-4'), 81.20 (C-3'), 83.12 (C-2'), 105.48 (C-1'), 109.38 ($Me_2\underline{C}$), 112.25 ($Me_2\underline{C}$), 120.59 (C-7), 127.75 (C-8), 169.77 (C=O).

7-Bromo-5-exo-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***249c***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.31 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.48, (s, 3H, CH_3), 2.08 (dt, 1H, *J* = 14.1, 3.3 Hz, H-6), 2.26-2.32 (m, 1H, H-6), 3.79 (dd, 1H, *J* = 3.1, 6.9 Hz, H-4), 3.95-3.98 (m, 3H, H-3', H-4', H-6'), 4.06-4.10 (m, 2H, H-5', H-6'), 4.22-4.24 (m, 1H, H-5), 4.58 (d, 1H, *J* = 3.6 Hz, H-2'), 5.05-5.06 (m, 1H, H-1), 5.81 (d, 1H, *J* = 3.6 Hz, H-1'), 6.46 (dd, 1H, *J* = 2.6, 6.4 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.51 (CH_3), 26.33 (CH_3), 26.83 (CH_3), 27.01 (CH_3), 33.62 (C-6), 49.05 (C-4), 67.83 (C-6'), 71.46 (C-5'), 72.47 (C-5), 80.51 (C-1), 81.58 (C-4'), 81.23 (C-3'), 83.61 (C-2'), 105.57 (C-1'), 109.24 ($Me_2\underline{C}$), 112.11 ($Me_2\underline{C}$), 123.86 (C-7), 127.30 (C-8), 169.41 (C=O).

LRMS (ESI^+): 480.0 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{19}H_{29}O_8N^{79}Br$ $[M+NH_4]^+$: 478.1071; found: 478.1069.

Cycloaddition between **221** & **226**

A solution of compound **221** (906 mg, 2.42 mmol, 2 eq.) and compound **226** (187 mg, 1.21 mmol, 1 eq.) in DCM (1 mL) was heated for 6 days at 45 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (5:1) to give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **250a/b** (351 mg, 55%) as a yellow oil.

*5-Endo-(β-D-glucose-1',2',3',4'-tetraacetate)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester **250a***

IR: (thin film) 2955, 2944, 1757, 1437, 1368, 1222, 1077, 1038, 981 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.67 (dt, 1H, *J* = 14.3, 1.72 H-6), 1.99 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.03 (s, 3H, CH_3, 2.56 (ddd, 1H, *J* = 3.6, 7.7, 14.3 Hz, H-6), 3.52 (dd, 1H, *J* = 5.7, 11.9 Hz, H-6'), 3.59-3.61 (m, 1H, H-6'), 3.63-3.66 (m, 1H, H-5'), 3.93 (s, 3H, OCH_3), 4.42 (dt, 1H, *J* = 7.6, 1.2 Hz, H-5), 4.95 (t, 1H, *J* = 9.5 Hz, H-4'), 5.06 (t, 1H *J* = 8.3 Hz, H-2'), 5.18 (t, 1H, *J* = 9.5 Hz, H-3'), 5.22-5.24 (m,

1H, H-1), 5.63 (d, 1H, *J* = 8.3 Hz, H-1'), 6.61 (dd, 1H, *J* = 5.2, 7.7 Hz, H-7), 6.74 (dt, 1H, *J* = 7.7, 1.3 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.70 (CH_3), 20.75 (CH_3), 20.79 (CH_3), 20.96 (CH_3), 36.00 (C-6), 53.24 (OCH_3), 61.73 (C-4) 68.27 (C-4'), 69.42 (C-6'), 70.23 (C-2'), 72.95 (C-3'), 74.31 (C-1), 74.55 (C-5'), 74.63 (C-5), 91.67 (C-1'), 129.64 (C-8), 130.87 (C-7), 167.66 (C=O), 168.72 (C=O), 169.36 (C=O), 169.42 (C=O), 169.47 (C=O), 170.24 (C=O).

5-Endo-(β-D-glucose-1',2',3',4'-tetraacetate)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester ***250b***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.72 (dt, 1H, *J* = 1.72, 14.09 H-6), 1.99 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.60 (ddd, 1H, *J* = 3.6, 7.7, 14.09 Hz, H-6), 3.47 (dd, 1H, *J* = 5.5, 11.7 Hz, H-6'), 3.57-3.61 (m, 1H, H-6'), 3.63-3.66 (m, 1H, H-5'), 3.91 (s, 3H, OCH_3), 4.42 (dt, 1H, *J* = 1.2, 7.6 Hz, H-5), 4.99 (t, 1H, *J* = 9.45 Hz, H-4'), 5.08-509 (m, 1H, H-2'), 5.18 (t, 1H, *J* = 9.5 Hz, H-3'), 5.22-5.24 (m, 1H, H-1), 5.62 (d, 1H, *J* = 8.3 Hz, H-1'), 6.60 (dd, 1H, *J* = 5.2, 7.7 Hz, H-7), 6.70 (dt, 1H, *J* = 1.2, 7.7 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.70 (CH_3), 20.75 (CH_3), 20.80 (CH_3), 20.86 (CH_3), 35.93 (C-6), 53.15 (OCH_3), 62.00 (C-4), 68.17 (C-4'), 69.42 (C-6'), 70.31 (C-2'), 73.13 (C-3'), 7352 (C-1), 74.00 (C-5), 74.65 (C-5'), 91.81 (C-1'), 129.73 (C-8), 130.83 (C-7), 167.77 (C=O), 169.15 (C=O), 169.36 (C=O), 169.52 (C=O), 169.58 (C=O), 170.32 (C=O).

LRMS (ESI^+): 546.0 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{23}H_{32}O_{14}N$ $[M+NH_4]^+$: 546.1817; Found: 546.1821.

Cycloaddition between **221** & **227**

A solution of compound **221** (0.707 g, 1.89 mmol, 2 eq.) and compound **227** (0.158 g, 0.944 mmol, 1 eq.) in DCM (1 mL) was heated for 6 days at 80 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. ^1H-NMR analysis of the crude reaction mixture indicated the presence of two cycloadducts in a 1:1 ratio. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (5:1) to give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **251a/b** (459 mg, 90%) as a yellow oil.

5-Endo-(β-D-glucose-1',2',3',4'-tetraacetate)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid ethyl ester ***251a/b***

IR: (thin film) 3025, 2943, 1757, 1638, 1368, 1300, 1219, 1077, 1038, 912 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.23 (t, 3H, *J* = 7.2 Hz, OCH_2CH_3), 1.61 (dd, 1H, *J* = 6.4, 15.0 Hz, H-6), 2.00 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), (ddd, 1H, *J* = 4.5, 7.9, 15.0 Hz, H-6), 3.47 (dd, 1H, *J* = 4.6, 11.6 Hz, H-6'), 3.59-3.64 (m, 1H, H-6'), 3.69-3.71 (m, 1H, H-5'), 4.04 (dd, 1H, *J* = 3.3, 16.0 Hz, H-4), 4.06-4.08 (m, 1H, H-5), 4.27-4.31 (m, 2H, OCH_2CH_3), 5.00-5.06 (m, 1H, H-4'), 5.07-5.10 (m, 1H, H-2'), 5.21 (t, 1H, *J* = 9.5 Hz, H-3'), 5.65 (t, 1H, *J* = 8.4 Hz, H-1'), 5.67-5.69 (m, 1H, H-1), 7.17-7.19 (m, 1H, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 14.31 (OCH_2CH_3), 20.71 (CH_3), 20.75 (CH_3), 20.77 (CH_3), 20.96 (CH_3), 34.82 (C-6), 47.57(C-4), 61.52 (OCH_2CH_3), 67.60 (C-6'), 68.54 (C-4'), 70.21 (C-2'), 72.65 (C-5), 72.92 (C-3'), 73.43 (C-1), 74. 09 (C-5'), 91.80 (C-1'), 136.36 (C-7), 137.80 (C-8), 169.25 (C=O), 169.38 (C=O), 169.41 (C=O), 169.96 (C=O), 170.25 (C=O), 170.52 (C=O).

LRMS (ESI^+): 560.1 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{24}H_{34}O_{14}N$ $[M+NH_4]^+$: 560.1974; Found: 560.1976.

Cycloaddition between **221** & **228**

A solution of compound **221** (2.64 g, 7.04 mmol, 20 eq.) and compound **228** (123 mg, 0.704 mmol, 1 eq.) in DCM (1 mL) and a few drops of Et_3N was heated for 6 days at 100 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. ^{1}H-NMR analysis of the crude reaction mixture indicated the presence of two cycloadducts in a 1:1 ratio. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (5:1) to give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **252a/b** (96 mg, 25%) as a yellow oil.

4-Bro-5-endo-(β-D-glucose-1',2',3',4'-tetraacetate)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***250a/b***

IR: (thin film) 2940, 1758, 1432, 1368, 1219, 1074, 1038, 907 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.87 (dt, 1H, *J* = 1.2, 14.1 Hz, H-6), 1.99 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.10 (s, 3H, CH_3) (ddd, 1H, *J* = 2.4, 7.9, 14.1 Hz, H-6), 3.71 (td, 1H, *J* = 2.4, 10.0 Hz, H-5') 3.79 (dd, 1H, *J* = 2.1, 10.0 Hz, H-6'), 3.95 (d, 1H, *J* = 7.7 Hz, H-5), 3.97 (dd, 1H, *J* = 3.4, 9.8 Hz, H-6'), 5.06-5.13 (m, 2H, H-2', H-

4'), 5.19-5.22 (m, 2H, H-1, H-3'), 5.65 (d, 1H, *J* = 8.42 Hz, H-1'), 6.41 (d, 1H, *J* = 7.9 Hz, H-8), 6.47 (dd, 1H, *J* = 5.0, 7.9 Hz, H-7).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.72 (CH_3), 20.75 (CH_3), 20.99 (CH_3), 21.01 (CH_3), 37.57 (C-6), 64.61 (C-4), 68.09 (C-2'), 70.17 (C-4'), 70.22 (C-6'), 72.98 (C-1), 73.23 (C-3'), 73.94 (C-5'), 77.83 (C-5), 91.86 (C-1'), 130.65 (C-7), 135.49 (C-8), 167.67 (C=O), 169.13 (C=O), 169.43 (C=O), 170.24 (C=O), 170.34 (C=O).

LRMS (ESI^+): 566.0 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{21}H_{29}O_{12}N^{79}Br$ $[M+NH_4]^+$: 566.0868; Found: 566.0859.

Cycloaddition between **221** & **229**

A solution of compound **221** (1.65 g, 4.41 mmol, 10 eq.) and compound **229** (77 mg, 0.441 mmol, 1 eq.) in DCM (1 mL) and a few drops of Et_3N was heated for 6 days at 100 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. ^{1}H-NMR analysis of the crude reaction mixture indicated the presence of two cycloadducts in a 1:1 ratio. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N

(92:6:12)), eluting with petroleum ether/EtOAc (4:1) to give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **253a/b** (158 mg, 65%) as a yellow oil.

7-Bro-5-endo-(β-D-glucose-1',2',3',4'-tetraacetate)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***250a***

IR: (thin film) 3022, 2944, 1760, 1433, 1368, 1221, 1161, 1075, 1038, 910 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.73 (dt, 1H, *J* = 1.4, 14.3 Hz, H-6), 2.02 (CH_3), 2.02 (CH_3), 2.03 (CH_3), 2.03 (CH_3), 2.55 (ddd, 1H, *J* = 4.0, 7.9, 14.3 Hz, H-6), 3.47 (dd, 1H, *J* = 4.8, 11.3 Hz, H-6'), 3.60 (dd, 1H, *J* = 2.8, 6.4 Hz, H-6'), 3.72 (ddd, 1H, *J* = 2.8, 5.0, 10.0 Hz, H-5'), 3.87 (dd, 1H, *J* = 3.3, 6.4 Hz, H-4), 3.97-3.99 (m, 1H, H-5), 5.06-5.08 (m, 1H, H-1), 5.07-5.10 (m, 2H, H-2', H-4'), 5.21 (t, 1H, *J* = 9.5 Hz, H-3'), 5.65 (dd, 1H, *J* = 8.4, 11.3 Hz, H-1'), 6.41-6.44 (m, 1H, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.7 (CH_3), 20.81 (CH_3), 20.94 (CH_3), 21.18 (CH_3), 35.05 (C-6), 48.44 (C-4), 67.84 (C-6'), 68.52 (C-1), 70.23 (C-4'), 72.30 (C-5), 72.87 (C-3'), 74.10 (C-5'), 80.55 (C-2'), 91.78 (C-1'), 120.46 (C-7), 127.58 (C-8), 169.24 (C=O), 169.37 (C=O), 169.48 (C=O), 170.01 (C=O), 170.24 (C=O).

7-Bro-5-endo-(β-D-glucose-1',2',3',4'-tetraacetate)-2-oxa-bicyclo[2.2.2]oct-8-en-3-one ***250b***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.75 (dt, 1H, *J* = 1.4, 14.3 Hz, H-6), 2.02 (CH_3), 2.02 (CH_3), 2.03 (CH_3), 2.03 (CH_3), 2.55 (ddd, 1H, *J* = 4.0, 7.9, 14.3 Hz, H-6), 3.47 (dd, 1H, *J* = 4.8, 11.3 Hz, H-6'), 3.60 (dd, 1H, *J* = 2.8, 6.4 Hz, H-6'), 3.72 (ddd, 1H, *J* = 2.8, 5.0,

10.0 Hz, H-5'), 3.84 (dd, 1H, *J* = 3.3, 6.4 Hz, H-4), 3.97-3.99 (m, 1H, H-5), 5.04 (m, 1H, H-1), 5.07-5.10 (m, 2H, H-2', H-4'), 5.21 (t, 1H, *J* = 9.5 Hz, H-3'), 5.65 (dd, 1H, *J* = 8.4, 11.3 Hz, H-1'), 6.41-6.44 (m, 1H, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.68 (CH_3), 20.79 (CH_3), 20.94 (CH_3), 21.18 (CH_3), 35.22 (C-6), 48.44 (C-4), 68.00 (C-6'), 68.55 (C-1), 70.19 (C-4'), 72.18 (C-5), 72.89 (C-3'), 73.89 (C-5'), 80.55 (C-2'), 91.74 (C-1'), 120.46 (C-7), 127.64 (C-8), 169.24 (C=O), 169.34 (C=O), 169.45 (C=O), 170.05 (C=O), 170.24 (C=O).
LRMS (ESI$^+$): 571.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{21}H_{29}O_{12}N^{79}Br$ $[M+NH_4]^+$: 566.0868; Found: 566.0857.

Cycloaddition between **225** & **227**

A solution of compound **225** (705 mg, 1.13 mmol, 2 eq.) and compound **227** (95.2 mg, 0.567 mmol, 1 eq.) in DCM (1 mL) was heated for 6 days at 100 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. ^{1}H-NMR analysis of the unpurified reaction mixture indicated the presence of two cycloadducts in a 6:1.9:1 ratio. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (6:1) to give a give a mixture of 5-*endo*-1/5-*endo*-2 cycloadducts **254a/b** (334 mg, 75%) as a yellow oil and and 5-*exo*-1 cycloadduct **254c** (67 mg, 15%) as a yellow oil.

*5-Endo-(*2',3',4',6'-tetra-*O*-benzoyl-α-D-mannopyranose*)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester* ***254a/b***

IR: (thin film) 3065, 3028, 2984, 1765, 1727, 1638, 1450, 1265, 1105, 1069, 1029, 978 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.40 (t, 3H, *J* = 7.1 Hz, CH_3CH_2O), 1.87 (dt, 1H, *J* = 1.7, 14.4 Hz, H-6), 2.75 (ddd, 1H, *J* = 3.8, 8.1, 14.3 Hz, H-6), 4.18 (dd, 1H, *J* = 3.4, 6.2 Hz, H-4), 4.29 (ddd, 1H, *J* = 2.75, 4.8, 10.0 Hz, H-5'), 4.32-4.40 (m, 2H, MeCH_2O), 4.49 (dd, 1H, *J* = 5.0, 12.2 Hz, H-6'), 4.57 (d, 1H, *J* = 6.2 Hz, H-5), 4.67 (dd, 1H, *J* = 2.6, 12.2 Hz, H-6'), 5.23 (s, 1H, H-1'), 5.56 (dd, 1H, *J* = 1.7, 3.3 Hz, H-2'), 5.75-5.76 (m, 2H, H-1, H-3'), 6.05 (t, 1H, *J* = 10.1 Hz, H-4'), 7.32 (d, 1H, *J* = 6.0 Hz, H-8), 7.36 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.39-7.45 (m, H, Ar-H), 7.52 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.58 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.62 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.81 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.81 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.94 (d, 2H, *J* = 8.3 Hz, Ar-H), 8.04-8.06 (m, 4H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): 14.32 (*C*H_3CH_2O), 34.96 (C-6), 46.81(C-4), 61.77 (CH$_3$*C*H_2O), 62.87 (C-6'), 66.84 (C-4'), 68.46 (C-5), 69.62 (C-3'), 69.81 (C-5'), 70.27 (C-2'), 73.22 (C-1), 96.05 (C-1'), 128.52 (Ar-CH), 128.62 (Ar-CH), 128.71 (Ar-CH), 128.75 (Ar-C), 128.84 (Ar-CH, C-7), 128.91 (Ar-C), 129.07 (Ar-C), 129.67 (Ar-C), 129.77 (Ar-CH), 129.85 (Ar-CH), 129.96 (Ar-CH), 130.01 (Ar-CH), 133.44 (Ar-CH),

133.53 (Ar-CH), 133.73 (Ar-CH), 133.90 (Ar-CH), 137.12 (C-8), 162.15 (C=O), 165.55 (C=O), 165.62 (C=O), 165.64 (C=O), 166.18 (C=O), 169.61 (C=O).

LRMS (ESI^+): 813.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{44}H_{42}O_{14}N$ $[M+NH_4]^+$: 808.2600; Found: 808.2592.

*5-Exo-(*2',3',4',6'-tetra-*O*-benzoyl-α-D-mannopyranose*)-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester* ***254c***

IR: (thin film) 3068, 2959, 2928, 1769, 1727, 1602, 1450, 1265, 1103, 1070, 1027, 912 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.32 (t, 3H, *J* = 7.1 Hz, C*H*$_3$CH$_2$O), 2.24 (ddd, 1H, *J* = 1.7, 9.11, 14.3 Hz, H-6), 2.37 (dt, 1H, *J* = 3.3, 14.3 Hz, H-6), 4.05 (dd, 1H, *J* = 3.1, 6.7 Hz, H-4), 4.27 (q, 2H, *J* = 7.2 Hz, OC*H*$_2$CH$_3$), 3.31 (dt, 1H, *J* = 9.1, 3.1 Hz, H-5), 4.36-4.39 (m, 1H, H-5'), 4.45 (dd, 1H, *J* = 3.8, 12.2 Hz, H-6'), 4.76 (dd, 1H, *J* = 2.6, 12.2 Hz, H-6'), 5.31 (d, 1H, *J* = 1.4 Hz, H-1'), 5.66 (dd, 1H, *J* = 1.9, 3.3 Hz, H-2'), 5.67-5.69 (m, 1H, H-1), 5.87 (dd, 1H, *J* = 3.3 , 10.1 Hz, H-3'), 6.08 (t, 1H, *J* = 10.1 Hz, H-4'), 7.21 (dd, 1H, *J* = 2.2, 6.7 Hz, H-8), 7.37 (t, 4H, *J* = 7.6 Hz, Ar-H), 7.41-7.43 (m, 4H, Ar-H),

7.50-7.53 (m, 1H, Ar-H), 7.56-7.62 (m, 3H Ar-H), 7.83 (m, 3H, Ar-H), 7.96 (dd, 2H, *J* = 1.2, 8.4 Hz, Ar-H), 8.05-8.06 (m, 3H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): 14.32 (*C*$H_3$$CH_2O$), 34.61 (C-6), 46.97(C-4), 61.71 (CH_3*C*H_2O), 62.84 (C-6'), 66.97 (C-4'), 69.05 (C-5), 69.86 (C-3'), 69.91 (C-5'), 70.55 (C-2'), 73.28 (C-1), 96.37 (C-1'), 128.44 (Ar-CH), 128.60 (Ar-CH, C-7), 128.62 (Ar-CH), 128.71 (Ar-CH), 128.76 (Ar-C), 128.13 (Ar-C), 129.21 (Ar-C), 129.42 (Ar-C), 129.89 (Ar-CH), 129.95 (Ar-CH), 129.98 (Ar-CH), 130.08 (Ar-CH), 133.23 (Ar-CH), 133.32 (Ar-CH), 133.59 (Ar-CH), 133.70 (Ar-CH), 137.08 (C-8), 165.39 (C=O), 165.60 (C=O), 165.62 (C=O), 165.68 (C=O), 166.21 (C=O), 166.45 (C=O).

LRMS (ESI$^+$): 813 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{44}H_{42}O_{14}N$ $[M+NH_4]^+$: 808.2600; Found: 808.2599.

3'-(2-Hydroxy-5-hydroxymethyl-cyclohex-5-enyloxy)-glucose ***255***

Compound **264** (96 mg, 0.277 mmol) was dissolved in 90% aq. trifluoroacetic acid (10 mL) at 0 ^oC and stirred for 30 minutes at 0 ^oC. The reaction mixture was brought to room temperature and stirred for another 30 minutes. The solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography eluting with

DCM/MeOH (3:1, 2:1) to give an anomeric mixture of the *title compound* **255** (68 mg, 80 %) as a colourless oil.

$[\alpha]_D$ = 28.6 (*c* 0.53, MeOH).

IR: (thin film) 3416, 2890, 1682, 1439, 1206, 1143, 1038, 844, 804 cm^{-1}.

^1H NMR (MeOD, 600 MHz): δ 1.51-1.56 (m, 1H, H-3), 1.91 (dd, 1H, *J* = 8.8, 17.4 Hz, H-1), 2.35-2.39 (m, 2H, H-1, H-3), 3.21-3.31 (m, 2H, H-4' and H-5'), 3.46 (dd, 1H, *J* = 3.6, 5.8 Hz, β-anomer H-2'), 3.53-3.7 (2H, m, H-6'), 3.75-3.83 (m, 2H, H-3' and H-2), 4.01-4.05 (m, 2H, H-6), 4.27-4.31 (m, 1H, H-4), 4.45-4.48 (m, 2H, β-anomer H1' & α-anomer H-2'), 5.08 (d, 1H, *J* = 3.6 Hz, α-anomer H-1'), 5.67 (m, 1H, H-5a).

^{13}C NMR (MeOD, 150.9 MHz): δ 34.01 (C-3), 36.36 & 36.48 (C-1), 61.49 & 61.62 (C-6'), 63.43 (C-2), 63.60 & 63.76 (C-6), 69.03 & 69.11 (C-4'), 72.00 & 73.66 (C-2'), 74.54 & 74.83 (C-4), 76.09 & 76.86 (C-5'), 81.13 & 84.31 (C-3'), 92.61& 96.62 (C-1'), 123.69 & 123.77 (C-5a), 137.83 & 137.88 (C-5).

LRMS (ESI^+): 329.1 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{13}H_{26}NO_8$ $[M+NH_4]^+$: 324.1658; Found: 324.1660.

(2R, 4R, 5R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5a-ene-5,5-dicarboxylic acid allyl ester methyl ester ***256***

A solution of lithium prop-2-en-1-olate was freshly prepared by adding *n*-BuLi (1.06 mL, 1.06 mmol) dropwise to allyl alcohol (30 mL) at -78 ^{o}C under a nitrogen atmosphere. After the addition was complete the solution was warmed to room temperature and stirred for 30 minutes. The solution was cooled to 0 ^{o}C and was then added dropwise to a solution of compound **246a** (1.16 g, 2.6 mmol) in allyl alcohol (20 mL) maintained at 0 ^{o}C under a nitrogen atmosphere. The reaction mixture was stirred for 2 h at 0 ^{o}C and then quenched with saturated aq. NH_4Cl and extracted with DCM (4 x 80 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petrol/EtOAc (3:1) to give the *title compound* **256** (1.07 g, 82%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -80.3 (*c* 3.63, $CHCl_3$)

IR: (thin film) 3477, 2988, 2940, 2898, 1740, 1650, 1452, 1374, 1247, 1215, 1165, 1119, 1074, 1022 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.30 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.79 (ddd, 1H, *J* = 1.5, 10.0, 14.0 Hz, H-3), 2.57 (dt, 1H, *J* = 14.0, 4.6 Hz, H-3), 3.73 (s, 3H, OCH_3), 3.86 (dd, 1H, *J* = 5.9, 8.8 Hz, H-6'), 4.00 (dd, 1H, *J* = 6.0, 8.8 Hz, H-6'), 4.06-4.07 (m, 1H, H-3'), 4.08-4.09 (m, 1H, H-5'), 4.10-4.12 (m, 1H, H-4'), 4.50-4.52 (m, 1H, H-4), 4.57-4.59 (m, 2H, H-2, H-7), 4.60 (d, 1H, *J* = 3.7 Hz, H-2'), 4.64 (ddd, 1H, *J* = 1.4, 5.5, 13.4 Hz, H-7), 5.26 (dd, 1H, *J* = 1.4, 10.5 Hz, H-9), 5.28 (dt, 1H, *J* = 1.4, *J* = 17.2 Hz, H-9), 5.70 (d, 1H, *J* = 3.7 Hz, H-1'), 5.84 (ddd, 1H, *J* = 5.5, 10.5, 17.2 Hz, H-8), 5.94 (d, 1H, *J* = 10.3 Hz, H-1), 6.02 (d, 1H, *J* = 10.3 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.47 (CH_3), 26.27 (CH_3), 26.84 (CH_3), 26.88 (CH_3), 32.00 (C-3), 52.84 (OCH_3), 59.19 (C-5), 63.37(C-5'), 66.43 (C-7), 67.42 (C-6'), 72.22 (C-4), 74.02 (C-2), 79.02 (C-4'), 80.92 (C-3'), 80.96 (C-2'), 105.32 (C-1'), 109.07 (Me_2C), 111.91 (Me_2C), 118.90 (C-9), 122.68 (C-1), 131.19 (C-8), 134.52 (C-5a), 167.62 (C=O), 169.22 (C=O).

LRMS (ESI$^+$): 521.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{24}H_{38}NO_{11}$ $[M+NH_4]^+$: 516.2439 ; Found: 516.2439.

(2S,4R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5-ene-carboxylic acid methyl ester ***261***

A mixture of compound **256** (150 mg, 0.301 mmol), formic acid (9.1 μL), Et_3N (5.4 μL), PPh_3 (3.95 mg, 15 μmol), and $Pd(OAc)_2$ (0.79 mg, 3.52 x 10^{-3} mmol) in dioxane (1 mL) was heated at 100 ^oC for 18 hours in a sealed tube. The reaction mixture was stripped of solvent and volatiles under reduced pressure and 1 M hydrochloric acid (1 mL) was added. This mixture was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with saturated aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petrol/EtOAc (5:1) to give the *title compound* **261** (79 mg, 63%) as a white crystalline solid.

mp: 135-136 ^oC

$[\alpha]_D^{20}$ = -8.5 (*c* 0.96, $CHCl_3$).

IR: (KBr) 3499, 3434, 2992, 2926, 1710, 1447, 1374, 1264, 1209, 1163, 1072 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.38 (d, 1H, J = 13.2 Hz, H-3), 1.41 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.09 (ddd, 1H, *J* = 2.2, 9.8, 19.1 Hz, H-1), 2.35 (d, 1H, *J* = 13.2 Hz H-3), 2.65 (dt, 1H, *J* = 19.1, 5.5 Hz, H-1), 3.72 (s, 3H, OCH_3), 3.94 (dd, 1H, *J* = 5.5, 8.6 Hz, H-6'), 4.04-4.07 (m, 3H, H-3', H-4', H-6'), 4.08-4.11 (m, 1H, H-5'), 4.21-4.25 (m, 1H, H-4), 4.49 (m, 1H, H-2), 4.93 (d, 1H, *J* = 3.8 Hz, H-2'), 5.78 (d, 1H, *J* = 3.8 Hz, H-1'), 7.02 (dd, 1H, *J* = 2.41 5.5 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.51 (CH_3), 26.43 (CH_3), 26.98 (CH_3), 27.04 (CH_3), 35.10 (C-3), 35.43 (C-1), 51.82 (OCH_3), 67.84 (C-6'), 70.75 (C-4), 72.47(C-5'), 80.32

(C-4'), 81.17 (C-3'), 82.25 (C-2'), 105.61 (C-1'), 109.12 (Me_2C), 111.86 (Me_2C), 129.59 (C-5), 142.08 (C-5a), 166.17 (C=O).

LRMS (ESI^+): 437.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{34}NO_9$ $[M+NH_4]^+$: 432.2228 ; Found: 432.2227.

(2S,4R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-5-hydroxymethyl-cyclohex-5-en-2-ol ***263***

iBu_2Al-H (2.9 mL of a 1 M solution in hexane, 2.9 mmol, 6.3 eq.) was added dropwise to a solution of compound **261** (19 mg, 0.461 mmol) in dry toluene (2 mL) maintained at -78 ^oC under a dry nitrogen atmosphere. The reaction was monitored by TLC. After 3 hours the reaction mixture was quenched at -78 ^oC by addition of a 1 M solution of potassium sodium tartrate (5 mL) and then EtOAc (15 mL). The reaction mixture was warmed to room temperature and stirred for 30 minutes. The organic layer was separated and the aqueous phase was extracted with EtOAc (4 x 40 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petrol/EtOAc (2:1) to give the *title compound* **263** (156 mg, 88 %) as a pale yellow oil.

$[\alpha]_D^{20} = 3.3$ (*c* 1.45, $CHCl_3$).

IR: (thin film) 3402, 2988, 2932, 1454, 1375, 1334, 1218, 1163, 1072, 1017 cm^{-1}.

^1H NMR ($CDCl_3$): δ 1.31 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.53-1.58 (m, 1H, H-3), 1.97 (dd, 1H, *J* = 8.9, 17.5 Hz, H-1), 2.22 (dt, 1H, *J* = 13.2, 4.5 Hz, H-3), 2.44 (dt, 1H, *J* = 5.2, 17.5 Hz, H-1), 3.94 (dd, 1H, *J* = 5.7, 8.6 Hz, H-6'), 4.03-4.05 (m, 3H, H-4, H-4' H-6'), 4.06-4.09 (m, 3H, H-3', H-6, H-6), 4.21 (m, 1H, H-2), 4.26 (dd, 1H, *J* = 5.8, 13.9 Hz, H-5'), 4.62 (d, 1H, *J* = 3.6 Hz, H-2'), 5.76-5.77 (m, 1H, H-5a), 5.85 (d, 1H, *J* = 3.6 Hz, H-1').

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.48 (CH_3), 26.40 (CH_3), 26.95 (CH_3), 26.99 (CH_3), 34.40 (C-1), 35.54 (C-3), 64.30 (C-2), 65.01 (C-6), 67.63 (C-6'), 72.43 (C-4), 72.46 (C-5'), 79.90 (C-4'), 81.07 (C-3'), 82.75 (C-2'), 105.48 (C-1'), 109.18 (Me_2C),111.14 (Me_2C), 126.90 (C-5a), 136.12 (C-5).

LRMS (ESI^+): 409.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{19}H_{34}NO_8$ $[M+NH_4]^+$: 404.2279 ; Found: 404.2277.

(2S,4R)-4-(1',2'-O-isopropylidene-α-D-glucofuranose)-5-hydroxymethyl-cyclohex-5-en-2-ol ***264***

Compound **263** (128mg, 0.331 mmol) was dissolved in 90% aq. AcOH (10 mL) and stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography eluting with DCM/MeOH (20:1→10:1) to give the *title compound* **264** (103 mg, 90%) as colourless crystals.

mp: 151-153 ^{o}C

$[[\alpha]_D^{20} = 3.8$ (*c* 0.77, $CHCl_3$).

IR: (KBr) 3386, 3321, 3248, 2978, 2935, 2882, 1378, 1255, 1225 cm^{-1}.

^{1}H NMR (MeOD): δ 1.30 (CH_3), 1.44 (s, 3H, CH_3), 1.47 (dd, 1H, *J* = 4.0, 12.0 Hz, H-3), 1.91 (dd, 1H, *J* = 9.6, 17.2 Hz, H-1), 2.27 (d, 1H, *J* = 13.0 Hz, H-3), 2.37 (dt, 1H, *J* = 17.3, 5.2 Hz, H-1), 3.54 (dd, 1H, *J* = 6.0, 11.5 Hz, H-6'), 3.72 (dd, 1H, *J* = 2.1, 11.5 Hz, H-6'), 3.82-3.83 (m, 1H, H-5'), 3.92-3.96 (m, 2H, H-3', H-2), 4.05-4.06 (m, 3H, H-4', H-6, H-6), 4.21 (s, 1H, H-4), 4.69 (d, 1H, *J* = 3.4 Hz, H-2'), 5.74 (d, 1H, *J* = 4.64 Hz, H-5a), 5.81 (d, 1H, *J* = 2.9 Hz, H-1').

^{13}C NMR (MeOD, 150.9 MHz): δ 25.17 (CH_3), 25.78 (CH_3), 33.90 (C-1), 35.42 (C-3), 62.75 (C-6), 63.45 (C-2), 64.14 (C-6'), 68.56 (C-4), 70.99 (C-5'), 79.62 (C-4'), 79.89 (C-3'), 82.22 (C-2'), 105.30 (C-1'), 111.62 (Me_2C), 125.27 (C-5a), 136.69 (C-5).

LRMS (ESI^+): 369.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{16}H_{30}NO_8$ $[M+NH_4]^+$: 464.1966 ; Found: 464.1964.

(2S, 4S, 5S)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5a-ene-5,5-dicarboxylic acid allyl ester methyl ester ***265***

A solution of lithium prop-2-en-1-olate was freshly prepared by adding ^{n}BuLi (2.26 mL, 2.26 mmol) dropwise to allyl alcohol (20 mL) at -78 ^{o}C under a dry nitrogen atmosphere. After the addition was complete the solution was warmed to room temperature and stirred for 30 minutes. This solution was cooled to 0 ^{o}C and was then added dropwise to a solution of *endo* cycloadduct **246b** (996 mg, 2.26 mmol) in allyl alcohol (2 mL) maintained at 0 ^{o}C under a nitrogen atmosphere. The reaction mixture was stirred for 2.5 hours at 0 ^{o}C and then quenched with saturated aq. NH_4Cl and extracted with DCM (4 x 80 mL), The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by

flash chromatography eluting with petrol/EtOAc (2:1) to give the *title compound* **265** (966 mg, 86%) as a pale yellow oil.

$[\alpha]_D^{20} = 44.4$ (*c* 1.51, $CHCl_3$).

IR: (thin film) 3468, 2988, 2939, 2898, 1738, 1650, 1452, 1374, 1248, 1211, 1165, 1119, 1074, 1020 cm^{-1}.

^1H NMR (($CDCl_3$, 600 MHz): δ 1.30 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.91 (ddd, 1H, *J* = 1.6, 10, 13.9 Hz, H-3), 2.40 (dt, 1H, *J* = 14.0, 5.3 Hz, H-3), 3.77 (s, 3H, OCH_3), 3.85 (dd, 1H, *J* = 5.8, 8.6 Hz, H-6'), 3.95 (dd, 1H, *J* = 5.8, 8.6 Hz, H-6'), 4.07-4.12 (m, 3H, H-3', H-4', H-5'), 4.30-4.33 (m, 1H, H-4), 4.56 (d, 1H, *J* = 3.61 Hz, H-2'), 4.56-4.59 (m, 1H, H-7), 4.61-4.65 (m, 1H, H-7), 4.72-4.23 (m, 1H, H-2)**,** 5.23 (dd, 1H, *J* = 1.2, 10.5 Hz, H-9), 5.28 (dd, 1H, *J* = 1.2, 17.3 Hz, H-9), 5.83 (d, 1H, *J* = 3.6 Hz, H-1'), 5.84 (ddd, 1H, *J* = 5.7, 10.7, 17.3 Hz, H-8), 5.96 (dd, 1H, *J* = 1.4, 10.3 Hz, H-1), 6.03 (d, 1H, *J* = 10.1 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.41 (CH_3), 26.37 (CH_3), 26.77 (CH_3), 26.90 (CH_3), 35.55 (C-3), 52.88 (OCH_3), 59.06 (C-5), 63.78 (C-5'), 66. 41 (C-7), 66.77 (C-6'), 72.35 (C-4), 75.38 (C-2), 81.18 (C-4'), 81.62 (C-3'), 82.62 (C-2'), 104.95 (C-1'), 108.96 (Me_2C), 112.08 (Me_2C), 118.86 (C-9), 123.44 (C-1), 131.22 (C-8), 134.39 (C-5a), 167.71 (C=O), 168.42 (C=O).

LRMS (ESI^+): 521.9 $[M+Na]^+$.

HRMS(EI) calcd for $C_{24}H_{38}NO_{11}$ $[M+NH_4]^+$: 516.2439 ; Found: 516.2439.

(2R,4S)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5-ene-carboxylic acid methyl ester ***266***

A mixture compound **265** (430 mg, 86.3 μmol), formic acid (51 μL), triethylamine (171 μL), PPh_3 (20.5 mg, 78 μmol), and $Pd(OAc)_2$ (4.7 mg, 20.9 μmol) in dioxane (2.3 mL) was heated at 100 ^oC for 19 h in a sealed tube. The reaction mixture was stripped of solvent and volatiles under reduced pressure and 1 M hydrochloric acid (1 mL) was added. This mixture was extracted with DCM (3 x 50 mL) and the combined organic layers were washed with saturated aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petrol/EtOAc (2:1) to give the *title compound* **266** (235 mg, 66%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -60.4 (*c* 2.83, $CHCl_3$).

IR: (thin film) 3468, 2989, 2937, 1717, 1438, 1374, 1327, 1254, 1217, 1163, 1076 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.28 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.60 (dt, 1H, J = 13.0, 3.6 Hz, H-3), 2.06 (ddd, 1H, *J* = 2.2, 9.3, 19.2 Hz, H-1), 2.16 (dt, 1H, *J* = 13.0, 3.3 Hz, H-3), 2.65 (dt, 1H, *J* = 19.2, 5.3 Hz, H-1), 3.73 (s, 3H, OCH_3), 3.87 (d, 2H, *J* = 6.4 Hz, H-6', H-6'), 4.07-4.12 (m, 1H, H-2), 4.16 (dd,

1H, *J* = 3.1, 6.2 Hz, H-4'), 4.20 (q, 1H, *J* = 6.2 Hz, H-5'), 4.27 (d, 1H, *J* = 3.1 Hz, H-3'), 4.55 (d, 1H, *J* = 3.6 Hz, H-2'), 4.59 (t, 1H, *J* = 2.8 Hz, H-4), 5.85 (d, 1H, *J* = 3.6 Hz, H-1'), 6.96 (dd, 1H, *J* = 2.6, 5.2 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.33 (CH_3), 26.40 (CH_3), 26.71 (CH3), 26.93 (CH3), 35.30 (C-3), 38.73 (C-1), 51.92 (OCH_3), 63.71(C-2), 66.59 (C-6'), 71.68 (C-4), 72.84 (C-5'), 81.14 (C-4'), 82.24 (C-3'), 83.27 (C-2'), 105.19 (C-1'), 108.60 (Me_2C), 111.94 (Me_2C), 130.33 (C-5), 141.36 (C-5a), 166.68 (C=O).

LRMS (ESI$^+$): 437.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{34}O_9Na$ ($[M+Na]^+$: 437.1782; Found: 437.1788.

(2R,4S)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-5-hydroxymethyl-cyclohex-5-en-2-ol ***267***

iBu_2Al-H (2.7 mL, of a 1 M solution in hexane, 2.7 mmol, 6.9 eq.) was added dropwise to a solution of compound **266** (162 mg, 0.391 mmol) in dry toluene (2 mL) maintained at -78 ^{o}C under a dry nitrogen atmosphere. The reaction was monitored by TLC. After 3 hours the reaction mixture was quenched at -78 ^{o}C by addition of a 1 M solution of potassium sodium tartrate (5 mL) and then EtOAc (10 mL). The reaction mixture was

warmed to room temperature and stirred for 30 minutes. The organic layer was separated and the aqueous phase was extracted with EtOAc (4 x 40 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petrol/EtOAc (1:2) to give the *title compound* **267** (122 mg, 81%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -57.6 (*c* 1.395, $CHCl_3$).

IR: (thin film) 3414, 2988, 2936, 1454, 1375, 1329, 1217, 1163, 1073, 1020 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.79 (ddd, 1H, *J* = 4.3, 10.5, 13.4 Hz, H-3), 2.00 (dd, 1H, *J* = 8.6, 16.7 Hz, H-1), 2.19 (dt, 1H, *J* = 13.4, 3.3 Hz, H-3), 2.44 (dt, 1H, *J* = 16.7, 5.2 Hz, H-1), 3.54 (t, 1H, *J* = 6.9 Hz, OH), 3.94 (dd, 1H, *J* = 6.9, 12.0 Hz, H-6), 4.06-4.11 (m, 4H, H-2, H-3', H-4', H-6'), 4.15 (dd, 1H, *J* = 6.2, 8.9 Hz, H-6'), 4.34-4.37 (m, 2H, H-5', H-6), 4.39 (t, 1H, *J* = 3.6 Hz, H-4), 4.57 (d, 1H, *J* = 3.6 Hz, H-2'), 5.67-5.68 (m, 1H, H-5a), 5.88 (d, 1H, *J* = 3.6 Hz, H-1').

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.08 (CH_3), 26.45 (CH_3), 27.04 (CH3), 27.09 (CH_3), 34.77 (C-1), 37.24 (C-3), 64.27 (C-2), 64.52 (C-6), 67.97 (C-6'), 73.15 (C-5'), 75.12 (C-4), 81.67 (C-4'), 82.69 (C-3'), 84.84 (C-2'), 105.48 (C-1'), 109.96 ($Me_2\underline{C}$), 112.10 ($Me_2\underline{C}$), 124.98 (C-5a), 137.73 (C-5).

LRMS (ESI^+): 409.3 [MNa^+].

HRMS (ESI) calcd for $C_{19}H_{34}O_8N_1$ $[M+NH_4]^+$: 404.2279 ; Found: 404.2277.

3’-(2-Hydroxy-5-hydroxymethyl-cyclohex-5-enyloxy)-glucose ***268***

Compound **267** (135 mg, 0.349 mmol) was dissolved in 90% aq. AcOH (10 mL) and stirred at room temperature for 17 h. The reaction mixture was concentrated under reduced pressure. The crude product was dissolved in TFA/THF/H_20 (3:1:0.1, 4.1 mL) and stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography eluting with DCM/MeOH (10:1, 5:1) to give an anomeric mixture of the title compound **268** (74 mg, 69 %) as a colourless oil.

IR: (thin film) 3433, 22528, 1642, 1447, 1206, 1143, 1039, 843, 807 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ 1.51-1.56 (m, 1H, H-3), 1.89 (dd, 1H, *J* = 8.8, 17.4 Hz, H-1), 2.36-2.39 (m, 2H, H-1, H-3), 3.17-3.31 (m, 2H, H-4’ and H-5’), 3.34-3.46 (m, 1H, β-anomer H-2’), 3.58-3.68 (m, 2H, H-6’), 3.73-3.81 (m, 2H, H-3’ and H-2), 4.01-4.07 (m, 2H, H-6), 4.28-4.30 (m, 1H, H-4), 4.46-4.49 (m, 2H, β-anomer H1’ & α-anomer H-2’), 5.10 (d, H, *J* = 3.6 Hz, α-anomer H-1’), 5.67 (m, 1H, H-5a).

^{13}C NMR (MeOD, 150.9 MHz): δ 33.9 & 34.0 (C-3), 36.33 & 36.5 (C-1), 61.21 & 61.37 (C-6’), 63.44 & 63.72 (C-6), 71.07 (C-4’), 71.6 (C-2’), 73.6 (C-2), 74.63 & 74.81 (C-4), 76.25 & 76.31 (C-5’), 81.16 & 84.31 (C-3’), 92.94 & 97.13 (C-1’), 123.73 & 123.89 (C-5a), 137.88 (C-5).

LRMS (ESI^+): 329.1 [$M+Na^+$].

HRMS (ESI) calcd for $C_{13}H_{22}O_8Na$ $[M+Na]^+$: 329.1207; Found: 329.1213.

(R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-cyclohexa-1,5-dienecarboxylic acid methyl ester ***270a*** *&* ***270b***

A solution of compound (10.97 g, 38.3 mmol, 2 eq.) and compound **226** (2.95 g, 19.2 mmol, 1 eq.) in DCM (2 mL) was heated for 7 days at 100 ^oC in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (15:1) to give the *title compound* ***270a*** (5.12 g, 67%) as white crystalline solid and the *title compound* ***270b*** (1.9 g, 25%) as pale yellow oil.

(R)-4-(1',2':5',6'-Diisopropylidene-α-D-glucofuranose)-cyclohexa-1,5-dienecarboxylic acid methyl ester ***270a***

mp: 122-124 ^oC

$[\alpha]_D^{20}$ = +39.4 (*c* 0.337, $CHCl_3$).

IR: (KBr) 2986, 2986, 2937, 2896, 1712, 1576, 1444, 1379, 1337, 1258, 1218, 1069 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 6H, CH_3), 1.42 (s, 3H, 2 x CH_3), 1.49 (s, 3H, CH_3), 2.31 (dd, 1H, *J* = 6.5, 19.1 Hz, H-3), 2.95 (dd, 1H, *J* = 3.6, 19.1 Hz, H-3), 3.79 (s, 3H, OCH_3), 3.89 (dd, 1H, *J* = 6.2, 8.6 Hz**,** H-6'), 4.00 (dd, 1H, *J* = 6.2, 8.6 Hz, H-6'), 4.04 (d, 1H, *J* = 3.1 Hz, H-3'), 4.09 (dd, 1H, *J* = 2.9, 7.7 Hz, H-4'), 4.14 (dd, 1H, *J* = 6.2, 12.4 Hz, H-5'), 4.47 (d, 1H, *J* = 6.4 Hz, H-4), 4.77 (d, 1H, *J* = 3.6 Hz, H-2'), 5.80 (d, 1H, *J* = 3.6 Hz, H-1'), 6.19-6.20 (m, 2H, H-1, H-2), 7.23-7.24 (m, 1H, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.60 (CH_3), 26.39 (CH_3), 26.81 (CH_3), 26.97 (CH_3), 29.11 (C-3), 51.83 (OCH_3), 67.38 (C-6'), 67.49 (C-4), 72.19 (C-5'), 79.30 (C-4'), 81.14 (C-3'), 83.37 (C-2'), 105.49 (C-1'), 108.76 ($Me_2\underline{C}$), 111.71 ($Me_2\underline{C}$), 122.73 (C-1), 125.23 (C-5), 133.27 (C-2), 136.19 (C-5a), 167.14 (C=O).

LRMS (ESI^+): 419 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{28}O_8Na$ $[M+Na]^+$: 419.1676; Found: 419.1676.

(S)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-cyclohexa-1,5-dienecarboxylic acid methyl ester ***270b***

$[\alpha]_D^{20}$ = -15.8 (*c* 4.03, $CHCl_3$).

IR: (thin film) 2988, 2937, 2895, 1712, 1574, 1456, 1373, 1256, 1217, 1073, 1022 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.31 (s, 6H, CH_3), 1.32 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.46 (ddd, 1H, *J* = 2.2, 6.2, 19.3 Hz, H-3), 2.77 (ddd, 1H, *J* = 1.4, 5.2, 19.4 Hz, H-3), 3.79 (s, 3H, OCH_3), 3.88 (dd, 1H, *J* = 3.4, 8.6 Hz**,** H-6'), 3.89 (dd, 1H, *J* = 6.0, 10.0 Hz, H-6'), 4.14-4.18 (m, 3H, H-3', H-4', H-5'), 4.46 (d, 1H, *J* = 3.8 Hz, H-2'), 4.57 (d, 1H, *J* = 6.2 Hz, H-4), 5.84 (d, 1H, *J* = 3.8 Hz, H-1'), 6.20-6.27 (m, 2H, H-1, H-2), 7.27 (d, 1H, *J* = 5.5 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.48 (CH_3), 26.47 (CH_3), 26.741 (CH_3), 27.03 (CH_3), 31.65 (C-3), 52.02 (OCH_3), 66.74 (C-6'), 66.86 (C-4), 72.96 (C-5'), 79.83 (C-4'), 81.13 (C-3'), 83.45 (C-2'), 105.37 (C-1'), 108.60 ($Me_2\underline{C}$), 111.92 ($Me_2\underline{C}$), 123.12 (C-1), 125.75 (C-5), 133.07 (C-2), 136.50 (C-5a), 167.72 (C=O).

LRMS (ESI^+): 419.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{32}O_8N$ $[M+NH_4]^+$: 414.2122; Found: 414.2125.

5-*Endo*-butoxy-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-4-carboxylic acid methyl ester **275**

A solution of compound **226** (537 mg, 3.48 mmol) and butylvinyl ether (5 mL) was heated for 44 h at 60 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (5:1) to give the title compound **275** (680 mg, 77%) as a pale yellow oil.

IR: (thin film) 2957, 2925, 2868, 1755, 1739, 1438, 1351, 1280, 1095, 975 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.84 (t, 3H, *J* = 7.6 H-4'), 1.24 (sextet, 2H, *J* = 7.6 Hz, H-3'), 1.37-1.45 (m, 2H, H-2'), 1.65 (dt, 1H, *J* = 1.72, 13.8 Hz, H-6), 2.56 (ddd, 1H, *J* = 3.8, 7.6, 13.8 Hz, H-6), 3.29-3.33 (m, H, H-1'), 3.42-3.46 (m, H, H-1'), 3.88 (s, 3H, OCH_3), 4.33 (dt, 1H, *J* = 7.6, 1.2 Hz, H-5), 5.23 (ddd, 1H, *J* = 1.7, 3.6, 6.9 Hz, H-1), 6.56 (dd, 1H, *J* = 5.2, 7.7 Hz, H-7), 6.76 (dd, 1H, *J* = 0.9, 7.7 Hz, H-8).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 13.76 (C-4'), 19.16 (C-3'), 31.62 (C-2'), 35.28 (C-6), 52.94 (OCH_3), 61.48 (C-4), 69.98 (C-1'), 72.79 (C-5), 74.30 (C-1), 129.75 (C-8), 130.57 (C-7), 167.68 (C=O), 168.84 (C=O).

LRMS (ESI^+): 272.2 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{13}H_{22}O_5N$ $[M+NH_4]^+$: 272.1492; Found: 286.1490.

5-*Endo*-butoxy-3-oxo-2-oxa-bicyclo[2.2.2]oct-8-ene-7-carboxylic acid ethyl ester **277**

A solution of compound **227** (581 mg, 2.37 mmol) and butylvinyl ether (4 mL) was heated for 24 h at 100 ^{o}C in sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether/EtOAc (2:1) to give the title compound **277** (486 mg, 76%) as a yellow oil.

IR: (thin film) 2960, 1762, 1713, 1465, 1381, 1334, 1297, 1255, 1160, 1096, 1027, 910 cm^{-1}

^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, *J* = 7.4 H-4'), 1.26-1.32 (m, 5H, H-3', $OCH_2C\mathit{H}_3$), 1.47 (q, 2H, *J* = 6.9 Hz, H-2'), 1.58 (dt, 1H, *J* = 14.3, 1.6 Hz, H-6), 2.60 (ddd, 1H, *J* = 3.8, 7.7, 14.3 Hz, H-6), 3.33-3.37 (m, H, H-1'), 3.43-3.46 (m, H, H-1'), 4.04-4.05 (m, 1H, H-5), 4.09 (dd, 1H, *J* = 3.6, 6.2 Hz, H-5), 4.23-4.26 (m, 3H, $OC\mathit{H}_2CH_3$), 5.67 (ddd, 1H, *J* = 1.9, 3.8, 7.4 Hz, H-1), 7.19 (d, 1H, *J* = 6.0 Hz, H-8).

^{13}C NMR ($CDCl_3$): δ 13.76 (C-4'), 14.25 ($OCH_2\underline{C}H_3$), 19.29 (C-3'), 31.65 (C-2'), 34.95 (C-6), 47.64 (C-4), 61.37 ($O\underline{C}H_2CH_3$), 69.33 (C-1'), 71.48 (C-5), 73.45 (C-1), 136.14 (C-8), 138.07 (C-7), 162.23 (C=O), 170.83 (C=O).

LRMS (ESI$^+$): 286.2 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{14}H_{24}O_5N$ $[M+NH_4]^+$: 286.1649; Found: 286.1656.

Compounds **280a & 280b**

Compound **270a** (400 mg, 1.01 mmol) was dissolved in DCM/H_2O (3:1, 8 mL) and *m*CPBA (261 mg, 1.51 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with 1 M $NaHSO_4$ (5 mL) and extracted with dichloromethane (3 x 25 mL). The organic layer was washed with $NaHCO_3$ (15 mL) and dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (7:1, 5:1) to give compound **280b** (99 mg, 24%) as a white crystalline solid and compound **280a** (263 mg, 63%) as a white crystalline solid.

(1R, 2S, 4R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-7-oxa-bicyclo[4.1.0]hept-5-ene-5-carboxylic acid methyl ester ***280a***

mp: 95-97 ^oC.

$[\alpha]_D^{20}$ = +5.6 (*c* 0.125, $CHCl_3$).

IR: (KBr) 2987, 2926, 2854, 1721, 1373, 1260, 1217, 1165, 1073, 849 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.66 (dd, 1H, *J* = 5.0, 16.0 Hz, H-3), 2.84 (d, 1H, *J* = 16.0 Hz, H-3), 3.43 (t, 1H, *J* = 4.1 Hz, H-1), 3.64 (m, 1H, H-2), 3.78 (s, 3H, OCH_3), 3.90 (dd, 1H, *J* = 6.9, 8.4 Hz**,** H-6'), 4.06 (m, 1H, H-4), 4.13 (m, 2H, H-3', H-6'), 4.43 (m, 1H, H-5'), 4.56 (m, 1H, H-4), 4.76 (d, 1H, *J* = 3.6 Hz, H-2'), 5.80 (d, 1H, *J* = 3.6 Hz, H-1'), 7.41 (dd, 1H, *J* = 1.4, 4.3 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.39 (CH_3), 26.27 (C-3), 26.47 (CH_3), 26.95 (CH_3), 27.03 (CH_3), 45.36 (C-1), 52.22 (OCH_3), 55.70 (C-2), 67.74 (C-6'), 69.02 (C-4), 72.24 (C-5'), 80.72 (C-4'), 81.16 (C-3'), 82.89 (C-2')**,** 105.54 (C-1'), 108.95 ($Me_2\underline{C}$), 111.80 ($Me_2\underline{C}$), 132.29 (C-5), 140.25 (C-5a), 165.84 (C=O).

LRMS (ESI^+): 435.2 [$M+Na^+$].

HRMS (ESI) calcd for $C_{20}H_{32}O_9N$ [$M+NH_4$]$^+$: 430.2072 ; Found: 430.2072.

(1S, 2R, 4R)-4-(1',2':5',6'-Di-O-isopropylidene-α-D-glucofuranose)-7-oxa-bicyclo[4.1.0]hept-5-ene-5-carboxylic acid methyl ester ***280b***

mp: 116-118 ^{o}C.

$[\alpha]_D^{20}$ = -72.5 (*c* 1.335, $CHCl_3$).

IR: (KBr) 3076, 2989, 2937, 2889, 1696, 1574, 1418, 1304, 1261, 1075, 916 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 2.21 (ddd, 1H, *J* = 4.0, 5.7, 15.5 Hz, H-3), 2.40 (ddd, 1H, *J* = 2.2, 6.2, 15.5 Hz, H-3), 3.36 (t, 1H, *J* = 3.6 Hz, H-1), 3.51 (dt, 1H, *J* = 5.5, 2.2 Hz, H-2), 3.76 (s, 3H, OCH_3), 3.95 (dd, 1H, *J* = 5.5, 8.8 Hz**,** H-6'), 4.03 (d, 1H, *J* = 3.3 Hz, H-4'), 4.04-4.07 (m, 2H, H-3', H-6'), 4.22 (dt, 1H, *J* = 5.8, 8.4, H-5'), 4.29 (t, 1H, *J* = 6.0 Hz, H-4), 4.78 (d, 1H, *J* = 3.6 Hz, H-2'), 5.81 (d, 1H, *J* = 3.6 Hz, H-1'), 7.00 (d, 1H, *J* = 3.6 H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.75 (CH_3), 26.59 (CH_3), 27.22 (2 x CH_3), 28.31 (C-3), 46.33 (C-1), 50.31 (C-2), 52.37 (OCH_3), 67.71 (C-4), 67.86 (C-6'), 72.57 (C-5'), 81.26 (C-4'), 81.28 (C-3'), 82.60 (C-2'), 105.80 (C-1'), 109.30 ($Me_2\underline{C}$), 112.13 ($Me_2\underline{C}$), 135.54 (C-5), 136.58 (C-5a), 166.11 (C=O).
LRMS (ESI^+): 435.1 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{32}O_9N$ $[M+NH_4]^+$: 430.2072 ; Found: 430.2071.

4-(*β-D-Glucose-1',2',3',4'-tetraacetate*)-cyclohexa-1,5-dienecarboxylic acid methyl ester ***271a/b***

A solution of compound **221** (1.07 g, 2.86 mmol, 2 eq.) and compound **226** (220 mg, 1.43 mmol, 1 eq.) in DCM (1 mL) was heated for 3 days at 60 ^{o}C in a sealed tube. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (pre-washed with petrol/EtOAc/Et_3N (92:6:12)), eluting with petroleum ether\EtOAc (5:1) to give a mixture of 5-*endo*-1/5-*endo*-2 **250a** & **250b** (225 mg, 30%) as yellow oil and a mixture of compounds **271a**/**b** (194 mg, 28%) as a yellow oil.

Isomer ***271a***

IR: (thin film) 2951, 2879, 1758, 1644, 1437, 1369, 1221, 1038, 914 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.97 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.03(s, 3H, CH_3), 2.36-2.42 (dt 1H, *J* = 14.1, 1.7 Hz, H-3), 2.77 (dd, 1H, *J* = 5.3, 14.6 Hz, H-3), 3.53-3.57 (m, 1H, H-6'), 3.67 (dd, 1H, *J* = 3.1, 11.0 Hz, H-6'), 3.71 (dt, 1H, *J* = 3.4, H-5'), 3.77 (s, 3H, OCH_3), 4.40 (dd, 1H, *J* = 6.9, 14.6 Hz, H-4), 5.04 (t, 1H *J* = 9.5 Hz, H-2'), 5.13 (t, 1H, *J* = 9.5 Hz, H-4'), 5.18 (t, 1H, *J* = 9.6 Hz, H-3'), 5.66 (d, 1H, *J* = 8.3 Hz, H-1'), 6.14-6.18 (m, 1H, H-1), 6.21-6.24 (m, 1H, H-2), 7.20 (d, 1H, *J* = 5.5 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.66 (CH_3), 20.70 (CH_3), 20.78 (CH_3), 20.95 (CH_3), 30.52 (C-6), 51.84 (OCH_3), 61.50 (C-4), 66.67 (C-6'), 67.79 (C-4), 68.74 (C-4'), 70.44 (C-2'), 73.17 (C-3'), 73.74 (C-5'), 91.84 (C-1'), 122.58 (C-1), 125.26 (C-5), 133.52 (C-2), 136.05 (C-5a), 167.64 (C=O), 169.12 (C=O), 169.37 (C=O), 169.47 (C=O), 170.32 (C=O).

Isomer ***271b***

^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.03 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.11(s, 3H, CH_3), 2.36-2.42 (m 1H, *J* = 1.7, 14.1 H-3), 2.77 (dd, 1H, *J* = 5.3, 14.6 Hz, H-3), 3.53-3.57 (m, 1H, H-6'), 3.67 (dd, 1H, *J* = 3.1, 11.0 Hz, H-6'), 3.79 (s, 3H, OCH_3), 4.40 (dd, 1H, *J* = 6.9, 14.6 Hz, H-4), 5.07 (t, 1H *J* = 9.5 Hz, H-2'), 5.12 (t, 1H, *J* = 9.5 Hz, H-4'), 5.24 (t, 1H, *J* = 9.5 Hz, H-3'), 5.71 (d, 1H, *J* = 8.4 Hz, H-1'), 6.14-6.18 (m, 1H, H-1), 6.21-6.24 (m, 1H, H-2), 7.24 (d, 1H, *J* = 5.7 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 20.66 (CH_3), 20.70 (CH_3), 20.91 (CH_3), 21.14 (CH_3), 30.61 (C-6), 51.91 (OCH_3), 60.48 (C-4), 66.85 (C-6'), 68.06 (C-4), 68.62 (C-4'), 70.28 (C-2'), 73.14 (C-3'), 74.37 (C-5'), 91.76 (C-1'), 122.58 (C-1), 125.11 (C-5), 133.50 (C-2), 135.99 (C-5a), 167.57 (C=O), 169.04 (C=O), 169.40 (C=O), 170.18 (C=O), 170.69 (C=O).

LRMS (ESI^+): 502.1 $[M+NH_4]^+$.

HRMS (ESI) calcd for $C_{22}H_{32}O_{12}N$ $[M+NH_4]^+$: 502.1919; Found: 502.1917.

(1S,2S,4R)-1-Azido-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5-enecarboxylic acid methyl ester ***281***

A solution of compound **280a** (313 mg, 0.759 mmol) in DME/EtOH/H_2O (2:1:1, 8 mL) was cooled to 0 ^oC. Sodium azide (294 mg, 4.52 mmol) was added followed by NH_4Cl (240 mg, 4.49 mmol). The reaction mixture was stirred at 0 ^oC for 1 h and then stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with water (15 mL) and extracted with EtOAc (4 x 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (4:1) to give the *title compound* **281** (293 mg, 85%) as a yellow oil.

$[\alpha]_D^{20}$ = -72.5 (*c* 1.335, $CHCl_3$).

IR: (thin film) 3434, 2988, 2925, 2853, 2106, 1723, 1643, 1435, 1373, 1258, 1218, 1072 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 1.33 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.85 (ddd**,** 1H, *J* = 2.5, 3.5, 15.5 Hz, H-3), 2.33 (ddd, 1H, *J* = 1.6, 3.4, 15.5 Hz, H-3), 3.80 (s, 3H, OCH_3), 3.90 (br s, 1H, H-3'), 4.02 (dd, 1H, *J* = 3.8, 8.9 Hz, H-6'), 4.08 (dd, 1H, *J* =1.2, 6.0 Hz, H-6'), 4.09-4.13 (m, 3H, H-1, H-2, H-4'), 4.17-4.20 (m, 1H, H-5'), 4.48-4.49 (m, 1H, H-4), 5.07 (d, 1H, *J* = 3.8 Hz, H-2'), 5.82 (d, 1H, *J* = 3.8 Hz, H-1'), 7.00 (d, 1H, *J* = 5.3 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 24.81 (CH_3), 26.36 (CH_3), 27.04 (CH_3), 27.12 (CH_3), 27.75 (C-3), 52.27(OCH_3), 58.74 (C-1), 66.17 (C-2), 66.49 (C-4), 67.90 (C-6'), 72.57 (C-5'), 79.97 (C-4'), 81.18 (C-3'), 81.51 (C-2')**,** 105.89 (C-1'), 109.64 ($Me_2\underline{C}$), 111.95 ($Me_2\underline{C}$), 132.52 (C-5)**,** 135.18 (C-5a), 165.67 (C=O).

LRMS (ESI^+): 478.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{33}O_9N_4$ $[M+NH_4]^+$: 473.2242; Found: 473.2244.

(1R,2R,4R)-1-Azido-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-2-hydroxy-cyclohex-5-enecarboxylic acid methyl ester ***282***

A solution of compound **280b** (59 mg, 0.143 mmol) in DME/EtOH/H_2O (1:0.5:0.5, 2 mL) was cooled to 0 ^oC. Sodium azide (56 mg, 0.861 mmol) was added followed by NH_4Cl (45 mg, 0.841 mmol). The reaction mixture was stirred at 0 ^oC for 1 h and then stirred at 45 ^oC for 16 h. The reaction mixture was concentrated under reduced pressure, diluted with water (5 mL) and extracted with EtOAc (4 x 25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (3:1, 2:1) to give the *title compound* **282** (58 mg, 89 %) as colourless oil.

$[\alpha]_D^{20}$ = +112.6 (*c* 0.23, $CHCl_3$).

IR: (thin film) 3478, 2988, 2953, 2937, 2103, 1722, 1656, 1493, 1374, 1256, 1218, 1165, 1074 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.35 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.57 (ddd**,** 1H, *J* = 1.7, 3.3, 13.8 H-3), 2.33 (ddd, 1H, *J* = 2.4, 3.4, 13.8 Hz, H-3), 3.78 (s, 3H, OCH_3), 3.94 (dd, 1H, *J* = 2.2, 8.8 Hz, H-1), 3.97 (dd, 1H, *J* = 5.2, 8.8 Hz, H-6'), 4.04-4.05 (m, 2H, H-2, H-4'), 4.07 (d, 1H, *J* = 3.1 Hz, H-3'), 4.10 (dd, 1H, *J* = 6.2, 8.8 Hz, H-6'), 4.22-4.25 (m, 1H, H-5'), 4.48-4.49 (m, 1H, H-4), 4.93 (d, 1H, *J* = 3.6 Hz, H-2'), 5.81 (d, 1H, *J* = 3.6 Hz, H-1'), 6.82 (d, 1H, *J* = 2.2 Hz, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 25.48 (CH_3), 26.44 (CH_3), 27.03 (CH_3), 27.04 (CH_3), 33.58 (C-3), 52.27 (OCH_3), 64.69 (C-1), 67.55 (C-2) 67.94 (C-6'), 69.81(C-4), 72.42 (C-5'), 80.89 (C-4'), 81.17 (C-3'), 82.13 (C-2'), 105.66 (C-1'), 109.25 ($Me_2\underline{C}$), 111.98 ($Me_2\underline{C}$), 131.91 (C-5), 138.76 (C-5a), 165.29 (C=O).

LRMS (ESI^+): 477.9 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{20}H_{29}O_9N_3Na$ $[M+Na]^+$: 478.1796; Found: 478.1801.

(1S,2S,4R)-1-Azido-2-(tert-butyl-dimethyl-silanyloxy)-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-cyclohex-5-enecarboxylic acid methyl ester ***283***

tert-Butyldimethylsilyl chloride (449 mg, 2.98 mmol) and imidazole (203 mg, 2.98 mmol) were added to a solution of compound **281** (227 mg, 0.498 mmol, 1 eq.) in DCM (5 mL) and maintained at 0 ^{o}C for 30 min. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 24 h. The reaction mixture was cooled to 0 ^{o}C, quenched with water (10 mL) and extracted with DCM (4 x 50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (6:1) to give the *title compound* **283** (223 mg, 79%) as a yellow oil.

$[\alpha]_D^{20}$ = +1.5 (*c* 0.71, $CHCl_3$).

IR: (thin film) 2988, 2955, 2934, 2888, 2859, 2104, 1726, 1438, 1373, 1254, 1074 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.11 [s, 3H, $Si(CH_3)_2$*t*-Bu], 0.15 [s, 3H, $Si(CH_3)_2$*t*-Bu], 0.91 [s, 9H,$C(CH_3)_3$], 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.72 (dt**,** 1H, *J* = 12.7, 9.2 Hz, H-3), 2.51 (ddd, 1H, *J* = 3. 8, 6.5, 12.7 Hz, H-3), 3.59 (ddd, 1H, *J* = 3.8, 8.3, 16.2 Hz, H-2), 3.75 (s, 3H, OCH_3), 3.95 (dd, 1H, *J* = 5.0, 8.6 Hz, H-6'), 4.03-4.06 (m, 3H, H-1, H-4', H-6'), 4.08 (d, 1H, *J* = 2.9 Hz, H-3'), 4.19-4.23 (m, 1H, H-5'), 4.49 (dddd, 1H, *J* = 1.4, 3.0, 6.5, 10.5 Hz, H-4), 4.81 (d, 1H, *J* = 3.6 Hz, H-2'), 5.79 (d, 1H, *J* = 3.6 Hz, H-1'), 6.50-6.51 (m, 1H, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ -4.88 & -4.58 [$Si(\underline{C}H_3)_2$], 17.96 ($\underline{C}Me_3$), 25.46 (CH_3), 25.72 [3 x $SiC(\underline{C}H_3)_3$], 26.36 (CH_3), 26.98 (CH_3), 27.01 (CH_3), 37.19, (C-3), 52.07 (OCH_3), 65.24 (C-1), 67.52 (C-6'), 71.70 (C-2), 72.34 (C-4), 72.44 (C-5'), 80.97 (C-4'), 81.18 (C-3'), 82.38 (C-2'), 105.51 (C-1'), 109.02 ($Me_2\underline{C}$), 111.87 ($Me_2\underline{C}$), 134.18 (C-5), 137.23 (C-5a), 165.86 (C=O).

LRMS (ESI^+): 592.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{26}H_{47}O_9N_7Si$ $[M+NH_4]^+$: 587.3107; Found: 587.3115.

(1S,2S,4R)-1-Amino-2-(tert-butyl-dimethyl-silanyloxy)-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-cyclohex-5-enecarboxylic acid methyl ester ***284***

PPh_3 (171 mg, 0.654 mmol, 1.9 eq.) was added to a solution of compound **283** (196 mg, 0.344 mmol) in THF (10 mL) and water (0.1 mL). The reaction mixture was heated under reflux for 3 h, cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (4:1) to give the *title compound* **284** (168 mg, 90%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -61.4 (*c* 1.17, $CHCl_3$).

IR: (thin film) 2986, 2955, 2934, 2888, 2858, 1722, 1438, 1372, 1217, 1166, 1073 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ ^1H NMR ($CDCl_3$, 600 MHz): δ 0.11 [s, 3H, $Si(CH_3)_2$], 0.114 [s, 3H, $Si(CH_3)_2$], 0.91 [s, 9H, $SiC(CH_3)_3$], 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3),

1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.69 (dt, 1H, *J* = 9.1, 12.9 Hz, H-3), 2.49 (ddd, 1H, *J* = 3.6, 6.7, 12.9 Hz, H-3), 3.59 (ddd, 1H, *J* = 3.6, 8.3, 12.0 Hz, H-2), 3.50-3.51 (m, 1H, H-1), 3.74 (s, 3H, OCH_3), 3.95 (dd, 1H, *J* = 5.2, 8.6 Hz, H-6'), 4.03-4.08 (m, 3H, H-3', H-4', H-6'), 4.21-4.24 (m, 1H, H-5'), 4.52-4.56 (m, 1H, H-4), 4.83 (d, 1H, *J* = 3.6 Hz, H-2'), 5.80 (d, 1H, *J* = 3.6 Hz, H-1'), 6.66 (br s, 1H, H-5a).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ -4.43 & -4.05 [$Si(CH_3)_2$], 18.1 [$\underline{C}(CH_3)_3$], 25.53 (CH_3), 25.87 [3 x $C(\underline{C}H_3)_3$], 26.46 (CH_3), 27.04 (CH_3), 27.07 (CH_3), 37.8 (C-3), 51.92 (OCH_3), 55.88 (C-1), 67.51 (C-6'), 72.48 (C-5'), 73.03 (C-4), 74.51 (C-2), 80.94 (C-4'), 81.07 (C-3'), 82.40 (C-2'), 105.62 (C-1'), 109.01 ($Me_2\underline{C}$), 111.89 ($Me_2\underline{C}$), 132.13 (C-5), 142.89 (C-5a), 166.56 (C=O).

LRMS (ESI^+): 544.2 (MH^+).

HRMS (ESI) calcd for $C_{26}H_{46}O_9NSi$ $[M+NH_4]^+$: 544.2936; Found: 544.2945.

[(1S,2S,4R)-1-Azido-2-(tert-butyl-dimethyl-silanyloxy)-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-cyclohex-5-enyl]-methanol ***290***

$^{i}Bu_2Al$-H (3.5 mL, of a 1 M solution in toluene, 3.5 mmol, 13 eq.) was added dropwise to a solution of compound **283** (155 mg, 0.272 mmol) in dry toluene (2 mL) maintained at -78 ^{o}C under a dry nitrogen atmosphere. The reaction was monitored by TLC. After 5 hours the reaction mixture was quenched at -78 ^{o}C by addition of a 1 M solution of potassium sodium tartarate (5 mL) and then EtOAc (15 mL). The reaction mixture was warmed to room temperature and stirred for 30 mins. The organic layer was separated and the aqueous phase was extracted with EtOAc (4 x 50 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (5:1) to give the *title compound* **290** (121 mg, 82 %) as a pale yellow oil.

$[\alpha]_D^{20}$ = 9.1 (*c* 2.59, $CHCl_3$).

IR: (thin film): 3473, 2986, 2955, 2933, 2887, 2859, 2102, 1463, 1373, 1256, 1217, 1163, 1075, 1020, 941, 840 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.12 [s, 3H, $Si(CH_3)_2$], and 0.15 [s, 3H, $Si(CH_3)_2$], 0.91 [s, 9H, $SiC(CH_3)_3$], 1.32 (s, 6H, 2 x CH_3), 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.71 (dt, 1H, *J* = 12.7, 10.1 Hz, H-3), 1.95 (br s, 1H, OH), 2.51 (ddd, 1H, *J* = 3.6, 5.5, 12.7 Hz, H-3), 3.62 (ddd, 1H, *J* = 3.6, 8.3, 12.5 Hz, H-2), 3.92-3.94 (m, 1H, H-1), 3.96 (dd, 1H, *J* = 5.2, 8.6 Hz, H-6'), 4.02 (d, 1H, *J* = 2.9 Hz, H-3'), 4.04-4.10 (m, 4H, H-4', H-6', C*H*$_2$OH), 4.23 (dt, 1H, *J* = 8.1, 5.5 Hz, H-5'), 4.27-4.29 (m, 1H, H-4), 4.63 (d, 1H, *J* = 3.6 Hz, H-2'), 5.57 (m, 1H, H-5a), 5.88 (d, 1H, *J* = 3.6 Hz, H-1').

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ -4.76 & -4.50 [$Si(CH_3)_2$], 18.06 ($\underline{C}Me_3$), 25.44 (CH_3), 25.82 [3 x $C(\underline{C}H_3)_3$], 26.37 (CH_3), 26.94 (CH_3), 27.01 (CH_3), 37.53, (C-3) ,63.75

(CH$_2$OH), 65.93 (C-1), 67.52 (C-6'), 71.71 (C-2), 72.30 (C-4), 74.44 (C-5'), 80.41 (C-4'), 80.92 (C-3'), 82.75 (C-2'), 105.45 (C-1'), 109.16 (Me$_2$<u>C</u>), 112.22 (Me$_2$<u>C</u>), 124.99 (C-5a), 141.00 (C-5)**.**

LRMS (ESI$^+$): 564.2 (MNa$^+$).

HRMS (ESI) calcd for $C_{25}H_{47}O_8N_4Si$ $[M+NH_4]^+$: 559.3158; Found: 559.3164.

[(1S,2S,4R)-1-Amino-2-(tert-butyl-dimethyl-silanyloxy)-4-(1',2':5',6'-di-O-isopropylidene-α-D-glucofuranose)-cyclohexyl]-methanol ***291***

Pd/C (10% weight) (97 mg) was added to a stirred solution of compound **290** (323 mg, mmol) in EtOAc (10 mL). The reaction mixture was stirred for 21 hours under a hydrogen atmosphere. The solution was filtered through celite™ and the solvent was removed under reduced pressure to give the *title compound* **291** (quantitative yield) as a pale yellow oil.

$[\alpha]_D^{20} = 5.8$ (*c* 1.86, $CHCl_3$).

IR: (thin film) 3361, 3294, 2988, 2945, 2934, 2893, 2859, 1460, 1373, 1255, 1216, 1165, 1076, 1022, 961, 838 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.085 & 0.092 [s, 3H, $Si(CH_3)_2$], 0.88 [s, 9H, $SiC(CH_3)_3$], 1.29 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.29-1.30 (m, 1H, H-5a) 1.40 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.55 (q, 1H, *J* = 12.2 Hz, H-3), 1.94-1.96 (d, 1H, *J* = 13.4, H-5a), 2.13 (dt, 1H, *J* = 14.3, 4.3 Hz, H-3), 2.25-2.29 (m, 1H, H-5), 2.69 (t, 1H, *J* = 10.5 Hz, H-1), 3.22 (ddd, 1H, *J* = 4.3, 9.3, 15.1 Hz, H-2), 3.53 (dd, 1H, *J* = 6.0, 11.2 Hz, H-6), 3.73 (dt, 1H, *J* = 4.8, 11.9 Hz, H-4), 3.93-3.94 (m, 2H, H-3', H-6'), 3.96 (dd, 1H, *J* = 3. 8, 11.2 Hz, H-6), 4.00 (dd, 1H, *J* = 2.9, 8.6 Hz, H-4'), 4.06 (dd, 1H, *J* = 6.0, 8.6 Hz, H-6'), 4.17 (dt, 1H, *J* = 8.8, 5.3 Hz, H-5'), 4.89 (d, 1H, *J* = 3.6 Hz, H-2'), 5.85 (d, 1H, *J* = 3.6 Hz, H-1').

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ -4.46 & -3.91 [$Si(CH_3)_2$], 18.07 ($\underline{C}Me_3$), 25.43 (CH_3), 25.91 [3 x $C(\underline{C}H_3)_3$], 26.30 (CH_3), 26.86 (CH_3), 27.01 (CH_3), 31.18 (C-5a), 34.94 (C-3), 40.84 (C-5), 52.10 (C-1), 61.71 (C-6), 67.67 (C-6'), 72.33 (C-5'), 75.15 (C-2), 78.45 (C-4), 81.02 (C-4'), 81.17 (C-3'), 83.37 (C-2'), 105.38 (C-1'), 109.19 ($Me_2\underline{C}$), 112.11 ($Me_2\underline{C}$).

LRMS (ESI^+): 546.1 (MH^+).

HRMS (ESI) calcd for $C_{25}H_{48}O_8NSi$ $[M+H]^+$: 518.3144; Found: 518.3135.

(1S,2S,4R)-1-Azido-2-(tert-butyl-dimethyl-silanyloxy)-4-(1',2'-di-O-isopropylidene-α-D-glucofuranose-)5-hydroxymethyl-cyclohex-5-ene ***292***

Compound **283** (97 mg, 0.179 mmol) was dissolved in AcOH/H_2O/TFA (9:1:0.5) 10.5 mL) and stirred at room temperature for 4 h and then concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (3:1) to give the *title compound* **292** (82 mg, 92%) as a pale yellow oil.

$[\alpha]_D^{20}$ = -25.5 (*c* 0.38, $CHCl_3$).

IR: (thin film) 3430, 2953, 2931, 2890, 2858, 2102, 1463, 1376, 1256, 1217, 1163, 1075, 1016, 960 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ -0.14 & -0.15 [s, 3H, $Si(CH_3)_2$], 0.91 [s, 9H, $SiC(CH_3)_3$], 1.28 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.70 (dt, 1H, *J* = 9.8, 12.4 Hz, H-3), 2.51 (ddd, 1H, *J* = 3.6, 5.7, 12.4 Hz, H-3), 3.52 (dd, 1H, *J* = 5.7, 11.7 Hz, H-6'), 3.68 (ddd, 1H, *J* = 3.6, 8.3, 15.6 Hz, H-2), 3.69 (dd, 1H, *J* = 2.9, 11.5 Hz, H-6'), 3.75-3.78 (m, 1H, H-5'), 3.91-3.93 (m, 1H, H-1), 3.97 (d, 1H, *J* = 13.4 Hz, H-6), 4.04-4.06 (m, 2H, H-3', H-4'), 4.06-4.09 (m, 1H, H-6), 4.34-4.36 (m, 1H, H-4), 4.72 (d, 1H, *J* = 3.8 Hz, H-2'), 5.55-5.56 (m, 1H, H-5a), 5.82 (d, 1H, *J* = 3.8 Hz, H-1').

^{13}C NMR (MeOD, 150.9 MHz): δ -6.13 & -5.71 [Si(CH_3)$_2$], 17.53 ($\underline{C}Me_3$), 25.44 (CH_3), 25.82 [3 x C($\underline{C}H_3$)$_3$], 25.14 (CH_3), 25.72 (CH_3), 36.96, (C-3), 61.20 (C-6), 64.11 (C-6'), 66.18 (C-1), 68.35 (C-5'), 71.73 (C-2), 71.83 (C-4), 79.28 (C-4'), 79.54 (C-3'), 81.81 (C-2'), 105.31 (C-1'), 111.55 ($Me_2\underline{C}$), 122.44 (C-5a), 142.11 (C-5).

LRMS (ESI$^+$): 524.2 [M+Na]$^+$.

HRMS (ESI) calcd for $C_{22}H_{43}O_9N_4Si$ [M+NH_4]$^+$: 519.2845; Found: 519.2847.

(1S,2S,4R)-1-Amino-2-(tert-butyl-dimethyl-silanyloxy)-4-(1,2,:5,6-di-O-isopropylidene-α-D-glucofuranose)-cyclohexanecarboxylic acid methyl ester ***294***

Pd/C (10% weight) (26 mg) was added to a stirred solution of compound **283** (178 mg, 0.312 mmol) in EtOAc (10 mL). The reaction mixture was stirred for 16 hours under a hydrogen atmosphere. The solution was filtered through celite and the solvent was removed under reduced pressure to give the *title compound* **294** (quantitative yield) as a pale yellow oil.

$[\alpha]_D^{20}$ = +28.5 (*c* 4.83, $CHCl_3$).

IR: (thin film) 2986, 2953, 2935, 2889, 2858, 1735, 1458, 1373, 1256, 1216, 1076, 1021, 941, 838 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.078 & 0.083 [s, 3H, $Si(CH_3)_2$], 0.89 [s, 9H, $SiC(CH_3)_3$], 1.30-1.31 (m, 4H, H-3, CH_3), 1.33 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.99 (m, 3H, H-3, H-3, H-5a), 3.05-3.07 (m, 1H, H-5), 3.08-3.12 (m, 1H, H-1), 3.17 (ddd, 1H, *J* = 4.8, 8.9, 13.8 Hz, H-2), 3.68 (s, 3H, OCH_3), 3.67-3.69 (m, 1H, H-5'), 3.93 (dd, 1H, *J* = 5.8, 8.6 Hz, H-6'), 4.0 (d, 1H, *J* = 2.9 Hz, H-3'), 4.06-4.09 (m, 2H, H-4', H-6'), 4.18-4.22 (m, 1H, H-4), 4.46 (d, 1H, *J* = 3.6 Hz, H-2'), 5.83 (d, 1H, *J* = 3.6 Hz, H-1').

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 150.9 MHz): δ -4.46 & -3.87 [$Si(CH_3)_2$], 18.12 ($\underline{C}Me_3$), 25.50 (CH_3), 25.92 [3 x $C(\underline{C}H_3)_3$], 26.36 (CH_3), 26.88 (CH_3), 26.99 (CH_3), 31.53 (C-5a), 35.62 (C-3), 43.48 (C-5), 51.66 (C-1), 51.77 (OCH_3), 67.56 (C-6'), 72.69 (C-5'), 75.60 (C-2), 76.85(C-4), 81.19 (C-4'), 81.50 (C-3'), 83.82 (C-2'), 105.83 (C-1'), 109.10 ($Me_2\underline{C}$), 111.95 ($Me_2\underline{C}$), 172.91 (C=O).

LRMS (ESI^+): 546.1 $[M+H]^+$.

HRMS (ESI) calcd for $C_{26}H_{48}O_9NSi$ $[M+H]^+$: 546.3093; Found: 546.3085.

(1S,2S,4R)-2-(Tert-butyl-dimethyl-silanyloxy)-4-(1',2',:5',6'-di-O-isopropylidene-α-D-glucofuranose)-1-(4-phenyl-[1,2,3]triazol-1-yl)-cyclohex-5-enecarboxylic acid methyl ester ***296***

Compound **283** (68 mg, 0.119 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (4 mL), phenyl acetylene (36.5 mg, 0.357 mmol) was added followed by sodium ascorbate (0.036 mmol, 36 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.018 mmol, 7.1 mg in 100 µL of water). The reaction mixture was heated at 85 ^{o}C for 30 hours. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel, eluting with petroleum ether/EtOAc (3:1) to give the *title compound* **296** (79 mg, 99 %) as a pale yellow oil.

$[\alpha]_D^{20}$ = -5.0 (*c* 2.6, $CHCl_3$).

IR: (thin film) 2988, 2956, 2932, 2893, 2858, 1725, 1657, 1611, 1460, 1437, 1373, 1334, 1257, 1163, 1124, 1074, 957, 840 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ -0.40 & -0.15 [2 x s, 2 x 3H, $Si(CH_3)_2$], 0.77 [s, 9H, $SiC(CH_3)_3$], 1.35 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 1.91 (dt**,** 1H, *J* = 16.3, 9.5 Hz, H-3), 2.68 (ddd, 1H, *J* = 2.8, 6.4, 13.1 Hz, H-3), 3.77 (s,

3H, OCH_3), 4.00 (dd, 1H, *J* = 5.0, 8.8 Hz, H-6'), 4.05-4.13 (m, 3H, H-2, H-4', H-6'), 4.17 (d, 1H, *J* = 2.8 Hz, H-3'), 4.25-4.29 (m, 1H, H-5'), 4.72-4.74 (m, 1H, H-4), 4.89 (d, 1H, *J* = 3.4 Hz, H-2'), 5.05-5.07 (m, 1H, H-1), 5.84 (d, 1H, *J* = 3.4 Hz, H-1'), 6.82-6.83 (m, 1H, H-5a), 7.33-7.36 (m, 1H, Ar-H), 7.42-7.45 (m, 2H, Ar-H), 7.79 (s, 1H, triazole-H), 7.80-7.82 (m, 2H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ -5.59 & -4.88 [$Si(CH_3)_2$], 17.84 [$\underline{C}Me_3$], 25.60 [3 x $C(\underline{C}H_3)_3$ and CH_3], 26.44 (CH_3), 27.06 (CH_3), 27.11 (CH_3), 37.69, (C-3), 52.26 (OCH_3), 64.98 (C-1), 67.68 (C-6'), 71.60 (C-2), 72.46 (C-4), 72.52 (C-5'), 81.04 (C-4'), 81.44 (C-3'), 82.46 (C-2'), 105.61 (C-1'), 109.15 ($Me_2\underline{C}$), 112.03 ($Me_2\underline{C}$), 121.26 & 147.55 (triazole), 125.88 (2 x Ar-CH), 128.40 (Ar-CH), 129.03 (2 x Ar-CH), 130.48 (Ar-C), 134.82 (C-5), 135.52 (C-5a), 165.72 (C=O).

LRMS (ESI^+): 672.2 $[M+H]^+$.

HRMS (ESI) calcd for $C_{34}H_{50}O_9N_3Si$ $[M+NH_4]^+$: 672.3311; Found: 672.3321.

Methyl 6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyraoside **335**

tert-butylchlorodimethylsilane (8.53 g, 56.65 mmol) and catalytic amount of DMAP were added to a stirred solution of α-D-methylmannopyronnoside (10.1 g, 52.04 mmol) in pyridine (20 mL) at 0 ^oC. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by TLC [$MeOH/CHCl_3$ (9:1)]. After completion (approx. 4 hours), the reaction mixture was diluted with EtOAc (100 mL)

and washed with 3 % sulphuric acid (3 x 50 mL), saturated aq. $NaHCO_3$ (3 x 50 mL) and saturated brine (3 x 50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography, eluting with ether to give the title compound **335** (8.00 g, 52 %) as a white solid.

mp. 102.2-103.6 ^{o}C.

IR: (Thin film) 3512, 2934, 2857, 2249, 1463, 1405, 1389, 1361, 1334, 1253, 1197, 1112 cm^{-1}.

^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.1 [s, 6H, $Si(CH_3)_2$], 0.2 [s, 9H, $SiC(CH_3)_3$], 3.36 (s, 3H, OCH_3), 3.54-3.59 (m, 1H, H-5), 3.73 (t, 1H, *J* = 9.0 Hz, H-4), 3.79-3.80 (m, 2H, H-3 and H-6), 3.85-3.88 (m, 2H, H-2, H-6), 4.69 (d, 1H, *J* = 1.1 Hz, H-1).

^{13}C NMR ($CDCl_3$, 90.6 MHz) δ: -4.76 & -4.45 [$Si(CH_3)_2$], 18.54 [$\underline{C}Me_3$], 26.00 [$C(\underline{C}H_3)_3$], 54.92 (OCH_3), 65.13 (C-6), 67.1 (C-4), 70.46 (C-2), 71.70 (C-5), 73.32 (C-3), 100.43 (C-1).

Anal. Calcd. For $C_{13}H_{28}O_6Si$: C, 50.62, H, 9.15. Found: C, 51.85, H, 8.60.

LRMS (ESI^+): 331.2 $[M+Na]^+$.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-tert-butyldimethyl-silyl-α-D-mannopyranoside **337**

Sodium hydride (4.43 g, 38.4 mmol) was added portion wise to a solution of compound **335** (7.88 g, 25.6 mmol) in DMF (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. BnBr (10.93 mL, 30.6 mmol) was added to the mixture at 0 °C. The reaction mixture was stirred at room temperature for 4 hours. The mixture was diluted with EtOAc (100 mL) and washed with water (3 x 50 mL) and saturated aq. sodium chloride (3 x 50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc 20:1 to give the title compound **337** (7.34 g, 50 %) as a colourless oil.

$[\alpha]_D^{20}$ = +19.2 (*c* 0.515, $CHCl_3$).

IR: (thin film): 4057, 3063, 3030, 2927, 2855, 2336, 1948, 1869, 1807, 1605, 1586, 1496, 1454, 1361, 1325, 1251, 1207, 1107, 1061 cm^{-1}.

^1H NMR ($CDCl_3$, 360 MHz): δ 0.01 [m, 6H, $Si(CH_3)_2$], 0.83 [s, 9H, $SiC(CH_3)_3$], 3.29 (s, 3H, OCH_3), 3.50-3.52 (m, 1H, H-5), 3.70 (s, 1H, H-2), 3.76-3.79 (m, 2H, H-3 and H-6), 3.82-3.87 (m, 2H, H-6 and H-4),), 4.54-4.59 (m, 3H, $ArCH_2$), 4.62 (s, 1H, H-1) 4.66 (s, 2H, $ArCH_2$), 4.86 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 7.19-7.30 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 90.6 MHz): δ -4.86 & -4.70 [$Si(CH_3)_2$], 18.73 [$\underline{C}Me_3$], 26.35 [$C(\underline{C}H_3)_3$], 54.87 (OCH_3), 63.23 (C-6), 72.54, 72.93, 75.37 (3 x s, 3 x $ArCH_2$), 73.53 (C-3), 75.32 (C-4), 80.67 (C-5), 99.08 (C-1), 127.91-128.75 (Ar-CH), 138.92 (Ar-C), 139.06 (Ar-C), 139.19 (Ar-C).

LRMS (ESI^+): 601.3 $[M+Na]^+$.

Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **338**

1M sulphuric acid (1 mL) was added to a stirred solution of compound **337** (7.18 g, 12.7 mmol) in methanol (25 mL) at 0 ^oC. The reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aq. $NaHCO_3$ (3 x 50 mL), water (3 x 50 mL) and saturated aq. sodium chloride or formula (3 x 50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc 2:1 to give the title compound **338** (5.40 g, 92%) as a colourless oil.

$[\alpha]_D^{20} = +32.3$ (*c* 5.75, $CHCl_3$).

IR: (Thin film) 3478, 3063, 3030, 2927, 2246, 1952, 1878, 1810, 1725, 1604, 1496, 1452, 1363, 1322, 1280, 1208 cm^{-1}.

^{1}H NMR ($CDCl_3$, 360 MHz) δ: 2.52 (OH), 3.35 (s, 3H, OCH_3), 3.39-3.71 (m, 1H, H-5), 3.84 (dd, 1H, *J* = 4.0, 7.0 Hz, H-6), 3.90 (d, 1H, *J* = 3.0 Hz, H-2), 3.94 (dd, 1H, *J* = 3.0, 7.0 Hz, H-6), 3.98 (d, 1H, *J* = 3.0 Hz, H-3), 4.05 (t, 1H, *J* = 9.5 Hz, H-4), 4.7 (s, 2H, $ArCH_2$), 4.71, (s, 1H, H-1), 4.74-4.86 (m, 3H, $ArCH_2$), 5.01 (d, 3H, *J* = 11.0 Hz, $ArCH_2$), 7.37-7.47 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 90.6 MHz): δ 55.34 (OCH_3), 62.76 (C-6), 72.54 (C-5), 72.65, 73.40, 75.65 (3 x s, 3 x $ArCH_2$), 75.11 (C-2), 75.27 (C-4), 80.63 (C-3), 99.74 (C-1), 128.04-129.11 (Ar-CH), 138.67 (Ar-C), 138.88 (Ar-C), 138.92 (Ar-C).

LRMS (ESI^+): 487.3 $[M+Na]^+$.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-amino-α-D-mannopyranoside **339**

Pd/C (10% weight) (744 mg) was added to a stirred solution of compound **342** (1.35 g, 2.76 mmol) in methanol (10 mL). Hydrogen gas was bubbled for 1 hour. The reaction mixture was stirred for a further 12 hours under a hydrogen atmosphere. The solution was filtered through celite to give **339 (**997 mg, 79%) as a yellow syrup.

IR: (Thin film) 4057, 5650, 3386, 3087, 2916, 2334, 2102, 1951, 1877, 1810, 1723, 1669, 1605, 1586, 1494, 1453, 1364, 1320, 1286 cm^{-1},

^{1}H NMR ($CDCl_3$, 360 MHz): δ 1.16 (br, 2H, NH_2), 2.73 (dd, 1H, *J* = 6.2 Hz, H-6), 2.93 (dd, 1H, 1H, *J* = 2.5, 11.0 Hz, H-6), 3.18 (s, 3H, OCH_3), 3.70 (m, 1H, H-5), 3.65-3.70

(m, 2H, H-2 and H-4), 3.79 (dd, 1H, *J* = 3.0, 6.0 Hz, H-3), 4.53 (s, 2H, $ArCH_2$), 4.7 (s, 1H, H-1), 4.61 (m, 3H, $ArCH_2$), 4.83 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 7.14-7.27 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 90.6 MHz): δ 43.72 (C-6), 55.10 (OCH_3), 75.51, 73.30, 72.54 ($ArCH_2$), 73.68 (C-5), 75.09 (C-2), 76.29 (C-4), 80.88 (C-3), 99.54 (C-1), 127.75-128.66 (Ar-CH).138.97 (Ar-C), 138.91 (Ar-C), 138.78 (Ar-C).

LRMS (ESI^+): 464.42 $[M+H^{+]}$.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-toluenesufonyl-α-D-mannopyranoside **341**

TsCl (2.9 g, 15.2 mmol) was added portion wise to a solution of compound **338** (2.02 g, 4.35 mmol) in pyridine (20 mL) at 0 ^oC. The reaction mixture was stirred at room temperature for 12 hours, the solvent was removed under reduced pressure and residue was purified by flash chromatography, eluting with petroleum ether/EtOAc 4:1 to give the title compound **341** (2.36g, 88 %) as white crystals.

mp: 108.0-110.7 ^oC

IR: (KBr) 3029, 2920, 2853, 2353, 1593, 1496, 1454, 1363, 1190, 1177, 1097, 1068 cm^{-1}.

^{1}H NMR ($CDCl_3$, 360 MHz) δ: 2.43 (s, 3H, CH_3), 3.27 (s, 3H, OCH_3), 3.76 (t, 1H, *J* = 2.4 Hz, H-2), 3.81 (d, 1H, *J* = 9.0 Hz, H-3), 3.87 (dd, 1H, *J* = 5.0, H-5), 4.23 (dd, 1H, *J* = 2.4, 12.4 H-6), 4.30 (dd, 1H, *J* = 2.0, 12.4 Hz, H-6), 4.49 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 4.60 (s, 2H, $ArCH_2$), 4.68 (s, 1H, H-1), 4.70-4.75 (m, 2H, $ArCH_2$), 4.91 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 7.2 (m, 2H, Ar-H), 7.27-7.34 (m, 15H, Ar-H), 7.80 (d, 2H, *J* = 8.0 Hz, Ar-H).

^{13}C NMR ($CDCl_3$, 90.6 MHz) δ: 22.05 (Ar-Me), 55.30 (OCH_3), 69.65 (C-6), 70.32 (C-5), 74.45 (C-4), 74.66 (C-2), 75.41, 73.06, 72.43, (3s, 3 x $ArCH_2$), 80.46 (C-3), 99.24 (C-1), 128.30-128.80(Ar-CH), 130.15 (Ar-C), 133.41(Ar-C), 138.45-138.63, (Ar-CH), 145.0 (Ar-C).

Anal. Calcd. For $C_{35}H_{38}O_8S$: C, 70.11, H, 6.54. Found: C, 70.04, H, 6.39

LRMS (ESI^+): 641.3 $[M+H]^+$.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-azido-α-D-mannopyranoside **342**

Sodium azide (1.24 g, 19.1 mmol) was added to a stirred solution of compound **341** (2.36 g, 3.81 mmol) in DMF (10 mL). The reaction mixture was stirred at 90 ^{o}C for 4 hours. The reaction mixture was diluted with EtOAc (150 mL) and washed with water (4 x 50 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure to give the title compound **342 (**1.80 g, 97%) as a colourless syrup.

$[\alpha]_D^{20}$ = 42.1 (*c* 10.8, $CHCl_3$).

IR: (Thin film) 4058, 3649, 3327, 3063, 3030, 3004, 2915, 2334, 2098, 1952, 1877, 1810, 1726, 1605, 1586, 1496, 1453, 1363, 1285, 1208, 1062 cm^{-1}.

^1H NMR ($CDCl_3$, 360 MHz) δ: 3.48 (s, 3H, OCH_3), 3.57 (dd, 1H, *J* = 6.5, 12.7 Hz, H-6), 3.61 (dd, 1H, *J* = 2.4, 12.7 Hz, H-6), 3.90-3.93 (m, 1H, H-5), 3.96 (s, 1H, H-4), 4.05 (m, 2H, H-2, and H-3), 4.72 (s, 1H, H-1), 4. 73 (s, 2H, $ArCH_2$), 4.84-4.93 (m, 3H, $ArCH_2$), 5.12 (d, *J* = 11.0 Hz, $ArCH_2$), 7.33-7.56 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 90.6 MHz): δ 51.96 (C-6), 55.38 (OCH_3), 75.65, 73.21, 72.47 ($PhCH_2$), 71.98 (C-5), 74.83 (C-4), 75.90 (C-2), 80.43 (C-3), 99.45 (C-1), 128.89-128.07 (Ar-CH), 138.76 (Ar-C), 138.67 (Ar-C), 138.64 (Ar-C).

LRMS (ESI^+): 512.2 $[M+Na]^+$.

Methyl 2,3,4-tri-O-benzyl-6-deoxy-6-cyclohexylamine-α-D-mannopyranoside ***343***

Cyclohexanone (111 mg, 1.13 mmol) was added to a stirred solution of compound **339** (348 mg, 0.37 mmol) in DCM (15 mL) containing molecular sieves. The reaction mixture was refluxed for 4 hours, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium

borohyride was added in portions over 3 hours. The solution was filtered and the solvent was removed under reduced pressure. Th crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc (10:1) to give the *title compound* **343** (231 mg, 56%) as a yellow syrup.

$[\alpha]_D^{20} = +40.8$ (*c* 1.16, $CHCl_3$).

IR: (thin film) 3064, 3030, 2926, 2853, 1495, 1454, 1363, 1124, 1062, 969 cm^{-1}.

^1H NMR ($CDCl_3$, 360 MHz): δ 0.79 (m, 1H), 1.00 (t, 2H, *J* = 12.0 Hz, H_{axial}), 1.1 (m, 2H, H_{axial}), 1.52 (d, 1H, *J* = 5.0 Hz, H_{eq}), 1.63 (d, 2H, *J* = 12.0 Hz, H_{eq}), 1.75 (t, 2H, *J* = 13.0 Hz, H_{eq}), 2.33 (m, 1H, CNH), 2.68 (dd, 1H, *J* = 4.0, 12.4 Hz, H-6), 2.96 (dd, 1H, *J* = 2.0, 12.4 Hz, H-6), 3.23 (s, 3H, OCH_3), 3.65 (m, 1H, H-5), 3.71 (m, 1H, H-2), 3.75 (d, *J* = 9.0 Hz, H-4), 3.80 (dd, 1H, *J* = 3.0, 6.0 Hz, H-3), 4.50 (s, 2H, $ArCH_2$), 4.57 (s, 1H, H-1), 4.60 (m, 3H, $ArCH_2$), 4.86 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 7.19-7.30 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 25.45 and 26.60 (C-3'), 33.68 and 33.91 (C-2'), 48.40 (C-6), 55.27 (OCH_3), 56.94 (CHN), 71.27 (C-5), 72.64 ($ArCH_2$), 73.22 ($ArCH_2$), 75.47 ($ArCH_2$), 75.06 (C-2), 77.44 (C-4), 80.73 (C-3), 99.53 (C-1), 127.93-128.76 (Ar-CH), 138.74 (Ar-C), 138.98 (Ar-C), 139.06 (Ar-C).

LRMS (ESI^+): 546.3 $[M+H]^+$.

HRMS (ESI) calcd for $C_{34}H_{44}O_5N$ $[M+H]^+$: 546.3214; Found: 546.3201.

*Methyl 2,3,4-tri-O-benzyl-6-deoxy-6-Benzy-α-D-mannopyranoside **344***

Benzaldehyde (114 mg, 1.07 mmol) was added to a stirred solution of compound **339** (332 mg, 0.72 mmol) in DCM (15 mL) containing molecular sieves. The reaction mixture was refluxed for 4 hours, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 3 hours. The solution was filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc (10:1) to give the *title compound* **344** (352 mg, 89%) as a yellow syrup.

$[\alpha]_D^{20} = +16.3$ (*c* 4.67, $CHCl_3$).

IR: (thin film) 3063, 3031, 2917, 1612, 1454, 1364, 1111, 1065, 968 cm^{-1}.

^1H NMR ($CDCl_3$, 500 MHz): δ 2.71 (dd, 1H, *J*' = 7.6, 12.4 Hz, H-6'), 2.91 (dd, 1H, *J*' = 2.4, 12.4 Hz H-6), 3.30 (s, 3H, OCH_3), 3.59 (m, 1H, H-4), 3.61 (s, 1H, $ArCH_2N$), 3.70 (t, 1H, *J* = 2.0 Hz, H-2), 3.72 (d, 1H, *J* = 14.0 Hz, $ArCH_2N$), 3.80 (dd, 1H, *J* = 3.0, 6.0 Hz, H-5), 3.84 (m, 1H, H-3), 4.29 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 4.50 (s, 2H, $ArCH_2$), 4.60 (s, 1H, H-1), 4.63 (m, 3H, $ArCH_2$), 4.70 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 6.88 (m, 2H, Ar-H), 7.12-7.30 (m, 18H, Ar-H).

^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 54.89 (C-6), 55.48 (OCH_3), 59.25 ($ArCH_2N$), 72.51, 73.11, 75.18 ($ArCH_2$), 72.71 (C-5), 75.00 (C-2), 76.91 (C-4), 80.72 (C-3), 99.41 (C-1), 127.09-129.19 (Ar-CH), 138.77 (Ar-C), 138.82 (Ar-C), 138.94 (Ar-C), 140.32 (Ar-C).

LRMS (ESI^+): 554.2 $[M+H]^+$.

HRMS (ESI) calcd for $C_{35}H_{40}O_5N_1$ $[M+H]^+$: 554.2901; Found: 554.2907.

Methyl 2,3,4-tri-O-benzyl-6-deoxy-6-(4-dimethoxybenzylamine)-α-D-mannopyranoside ***345***

Anisaldehyde (163 mg, 1.20 mmol) was added to a stirred solution of compound **339** (371 mg, 0.8 mmol) in DCM (20 mL) containing molecular sieves. The reaction mixture was refluxed for 4 hours then filtered. The solvent was removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 3 hours. The solution was filtered and solvent was removed under reduced pressure. The crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc (5:1) to give the *title compound* **345** (388 mg, 83%) as a yellow syrup.

$[\alpha]_D^{20}$ = +35.1 (*c* 1.45, $CHCl_3$).

IR: (thin film) 3030, 2924, 2830, 1609, 1512, 1454, 1251, 1115, 1065, 968 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.80 (dd, 1H, *J* = 7.9, 12.0 Hz, H-6), 3.05 (dd, 1H, *J* = 2.4, 12.0 Hz, H-6), 3.37 (s, 3H, OCH_3), 3.76 (3H, OCH_3), 3.76 (s, 1H, ArCHN), 3.80 (m, 1H, ArCHN), 3.82 (d, 1H, *J* = 6.6 Hz, H-4), 3.87 (d, 1H, *J* = 2.9 Hz, H-5), 3.88 (m, 1H, H-2), 3.93 (d, 1H, *J* = 18.2 Hz, H-3,), 4.52 (d, 1H, *J* = 1.1 Hz, $ArCH_2$), 4.59 (s, 2H, $ArCH_2$), 4.69 (m, 2H, H-1, $ArCH_2$), 4.73 (d, 1H, *J* = 12.4 Hz, $ArCH_2$), 4.88 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 6.83 (d, 1H, *J* = 8.6 Hz, Ar-H), 7.17 (d, 1H, *J* = 6.8 Hz, Ar-H), 7.25-7.37 (m, 2H, Ar-H), 7.25-740 (m, 15H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 48.5 (C-6), 52.08 ($ArCH_2N$), 55.34, 55.44, (2 x OCH_3), 72.23, 73.07, 75.03 ($ArCH_2$), 69.3 (C-5), 74.58 (C-2), 76.45 (C-4), 80.16 (C-3), 99.24 (C-1), 127.70-128.75 (Ar-CH, Ar-C), 130.62 (Ar-CH), 138.25 (Ar-C), 138.34 (Ar-C), 138.44 (Ar-C)

LRMS (ESI^+): 584.3 $[M+H]^+$.

HRMS (ESI) calcd for $C_{36}H_{42}O_6N_1$ $[M+H]^+$: 584.3007; Found: 584.3008.

Methyl 2,3,4-tri-O-benzyl-6-deoxy-6-(3,4-dimethoxybenzylamine)-α-D-mannopyranoside ***346***

3,4-dimethoxybenzaldehyde (154 mg, 0.93 mmol) was added to a stirred solution of compound **339** (287 mg, 0.62 mmol) in DCM (20 mL) containing molecular sieves. The reaction mixture was refluxed for 4 hours, filtered and the solvent was removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 3 hours. The solution was filtered and solvent was removed under reduced pressure. The crude product was purified by flash chromatography, eluting with petroleum ether/EtOAc (3:1) to give the *title compound* **346** (233 mg, 61%) as a yellow syrup.

$[\alpha]_D^{20}$ = +33.8 (*c* 4.14, $CHCl_3$).

IR: (thin film) 3030, 3003, 2908, 2834, 1592, 1515, 1454, 1265, 1237, 1132, 1064, 1028, 968 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): δ 2.85 (dd, 1H, *J* = 7.2, 12.4 Hz, H-6), 3.01 (dd, 1H, *J* = 2.8, 12.4 Hz, H-6), 3.32 (s, 3H, OMe), 3.73 (d, 1H, *J* = 13.1 Hz, H-4), 3.78 (m, 1H, H-5), 3.80 (m, 2H, H-2, ArCHN), 3.85 (s, 3H, OCH_3), 3.86 (3H, OCH_3), 3.88 (m, 2H, H-3, ArCHN), 4.57 (d, 1H, *J* = 1.1 Hz, $ArCH_2$), 4.62 (d, 2H, *J* = 1.0 Hz, $ArCH_2$), 4.69 (d, 1H, *J* = 1.4 Hz, H-1), 4.72 (d, 1H, *J* = 12.4 Hz, $ArCH_2$), 4.78 (d, 1H, *J* = 12.3 Hz, $ArCH_2$), 4.91 (d, 1H, *J* = 11.0 Hz, $ArCH_2$), 6.79 (d, 1H, *J* = 7.9 Hz, Ar-H), 6.85 (dd, 1H, *J* = 2.0, 16.1 Hz, Ar-H), 6.91 (d, 1H, *J* = 2.1 Hz, Ar-H), 7.2-7.21 (m, 2H, Ar-H), 7.25-740 (m, 14H, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 50.16 (C-6), 53.67 ($ArCH_2N$), 54.91, 55.88, 55.91 (3 x OCH_3), 72.31, 72.95, 74.90 ($ArCH_2$), 71.01 (C-5), 74.72 (C-2), 76.82 (C-4), 80.38 (C-

3), 100.0 (C-1), 127.69-128.47 (Ar-CH), 133.03 (Ar-C), 138.40 (Ar-C), 138.60 (Ar-C), 138.63 (Ar-C), 140.05 (Ar-C), 149.00 (Ar-C).

LRMS (ESI^+): 614.4 $[M+H]^+$.

HRMS (ESI) calcd for $C_{37}H_{44}O_7N_1$ $[M+H]^+$: 614.3112; Found: 614.3106.

6-cyclohexylamine-6-deoxy-methyl-α-D-mannopyranoside ***351***

Pd/C (10% weight) (20 mg) was added to a stirred solution of compound **343** (155 mg, 0.13 mmol) in methanol (10 mL). The reaction mixture was stirred for 4 days under a hydrogen atmosphere. The solution was filtered through celite and washed with EtOAc. The solvent was removed under reduced pressure to give the *title compound* **351** (62 mg, 79%) as a colourless oil.

IR: (thin film) 3393, 2939, 2858, 1580, 1451, 1413, 1133, 1061, 966 cm^{-1}

^1H NMR (MeOD, 600 MHz): δ 1.18-1.26 (m, 1H, cy), 1.34-1.43 (m, 5H, cy), 1.70 (d, 1H, *J* = 12.2 Hz, cy), 1.86-1.90 (m, 2H, cy), 2.10-2.17 (m, 1H, cy), 3.06-3.09 (m, 1H, cy), 3.18 (dd, 1H, *J* = 8.4, 12.5 Hz, H-6), 3.29 (s, 3H, OCH_3), 3.38 (dd, 1H, *J* = 2.9, 12.5

Hz, H-6), 3.50 (t, 1H, *J* = 9.6 Hz, H-4), 3.65 (dd, 1H, *J* = 3.4, 18.7 Hz, H-3), 3.73 (m, 1H, H-5), 3.80 (dd, 1H, *J* = 1.8, 6.7 Hz, H-2), 4.67 (d, 1H, *J* = 1.8 Hz, H-1).

^{13}C NMR (MeOD, 150.9 MHz): δ 24.26 and 24.32 (C-3'), 24.79 (C-4'), 28.64 and 29.11 (C-2'), 57.69 (C-1'), 45.69 (OCH_3), 68.51 (C-4), 68.54 (C-2), 70.51 (C-3), 70.56 (C-5), 101.81 (C-1).

LRMS (ESI^+): 276.1 $[M+H]^+$, 298.1 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{13}H_{26}O_5N_1$ (MNH_4^+): 276.1805; Found: 276.1806.

6-O-Toluenesulfonyl-6-deoxy-methyl-α-D-mannopyranoside **352**

Methyl mannopyranoside (10.1 g, 52.01 mmol) was dissolved in pyridine (100 mL) and cooled to 0 ^oC, TsCl (14.49 g, 76.0 mmol) was added in portions . The reaction mixture was stirred at 0 ^oC for 8 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (25:1) to give the title compound **352** (13.1 g, 72 %) as a colourless syrup..

$[\alpha]_D^{20}$ = -101.3 (*c* 6.98, $CHCl_3$).

IR: (thin film) 3440, 2932, 2918, 1599, 1445, 1358, 1176, 1096, 1063, 980 cm^{-1}

^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.43 (s, 3H, CH_3), 3.29 (s, 3H, OCH_3), 3.67-3.75 (m, 3H, H-2, H-3, H-5), 3.89 (s, 1, H-4), 4.27 (d, 1H, *J* = 10.6 Hz, H-6), 4.32 (dd, 1H, *J* = 5.0, 10.6 Hz, H-6), 4.64 (s, 1H, H-1), 7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.2 Hz, Ar-H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 21.74 (Me), 55.13 (OCH_3) 69.68 (C-6), 67.09 (C-4), 70.10 (C-2), 70.57 (C-3), 71.10 (C-5), 101.03 (C-1), 128.11 (Ar-CH), 129.98 (Ar-CH), 132.69 (Ar-C), 145.08 (Ar-C).

LRMS (ESI^+): 349.4 $[M+H]^+$, 371.4 $[M+Na]^+$

6-Azido-6-deoxy-methyl-α-D-mannopyranoside **353**

Sodium azide (6.15 g, 94.6 mmol) was added portionwise to a stirred solution of compound **352** (11.72 g, 33.7 mmol) in DMF (100 mL). The reaction mixture was stirred at 90 ^{o}C for 5 hours. The solution was filtered to remove white solid and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography eluting with DCM/MeOH (10:1) to give the title compound **353** (7.1 g, 96 %) as a colourless syrup.

$[\alpha]_D^{20}$ = +71.1 (*c* 9.87, $CHCl_3$).

IR (Thin film): 3399, 3011, 2934, 2838, 2102, 1655, 1445, 1282, 1096, 965 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 3.38 (s, 3H, OCH_3), 3.4 (dd, 1H, *J* = 6.0, 8.4 Hz, H-6), 3.46 (dd, 1H, *J* = 2.4, 13.0 Hz, H-6), 3.54 (t, 1H, *J* = 9.5 Hz, H-4), 3.58-3.62 (m, 2H, H-3 and H-5), 3.78 (dd, 1H, *J* = 3.3, 7.0 Hz, H-2), 4.62 (d, 1H, *J* = 1.7 Hz, H-1).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.56 (C-6), 54.02 (OCH_3), 68.11 (C-4), 70.61 (C-2), 71.02 (C-3), 72.34 (C-5), 101.48 (C-1).

LRMS (ESI^+): 242.2 $[M+Na]^+$.

6-Amino-6-deoxy-methyl-α-D-mannopyranoside **354**

Pd/C (10% weight) (3.1 g) was added to a stirred solution of compound **353** (10.1 g, 46.1 mmol) in methanol (100 mL). The reaction mixture was stirred for 20 hours under a hydrogen atmosphere. The solution was filtered through celite and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography, eluting with DCM/MeOH (2:1) to give the title compound **354** (6.4 g, 72 %) as a colourless syrup.

$[\alpha]_D^{20}$ = +69.7 (*c* 1.71, MeOH).

IR: (thin film) 3362, 2997, 2923, 2839, 2507, 1568, 1451, 1384, 1199, 1134, 1061, 972 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 2.79 (dd, 1H, *J* = 7.0, 13.4 Hz, H-6'), 2.97 (dd, 1H, *J* = 2.7, 13.3 Hz, H-6), 3.35 (s, 3H, OCH_3), 3.40-3.43 (m, 1H, H-5), 3.50 (t, 1H, *J* = 9.6 Hz, H-4), 3.63 (dd, 1H, *J* = 3.4, 9.5 Hz, H-3), 3.77 (dd, 1H, *J* = 1.8, 3.3 Hz, H-2), 4.62 (d, 1H, *J* = 1.4 Hz, H-1),

^{13}C NMR (MeOD, 150.9 MHz): δ 42.24 (C-6), 54.01 (OCH_3), 68.50 (C-4), 70.85 (C-2), 71.14 (C-3), 72.74 (C-5), 101.50 (C-1).

LRMS (ESI^+): 194.2 $[M+H]^+$, 216.2 $[M+Na]^+$.

6-Benzyl-6-deoxy-methyl-α-D-mannopyranoside ***356***

Benzaldehyde (260 mg, 14.8 mmol) was added to a stirred solution of compound **354** (316 mg, 1.64 mmol), MeOH (20 mL) containing molecular sieves. The reaction mixture was refluxed for 24 hours, filtered and the solvent removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 4 hours. The solution was treated with 2M NaOH (20 mL), filtered and the solvent removed under reduced pressure. The residue

was purified by flash chromatography, eluting with DCM/MeOH (10:1) to give the *title compound* **356** (295 mg, 63%) as a colourless syrup.

$[\alpha]_D^{20}$ = +45 (*c* 3.02, MeOH).

IR: (thin film) 3321, 2928, 2835, 1597, 1551, 1452, 1385, 1133, 1063, 978 cm^{-1}.

^1H NMR (D_2O, 600 MHz): δ 2.77 (dd, 1H, *J* = 9.7, 12.5 Hz, H-6), 3.00 (d, 1H, *J* = 12.5 Hz, H-6), 3.14 (s, 3H, OCH_3), 3.34 (t, *J* = 9.7 Hz, H-4), 3.53-3.56 (m, 2H, H-3, H-5), 3.71 (s, 1H, H-2), 3.77 (s, 2H, $ArCH_2$), 4.51 (s, 1H, H-1), 7.18-7.23 (m, 4H, Ar-H), 7.66 (d, 1H, *J* = 7.4 Hz, Ar-H).

^{13}C NMR (D_2O, 150.9 MHz): δ 48.34 (C-6), 51.87 (CH_2Ar), 55.04 (OCH_3), 68.59 (C-4), 69.11 (C-2), 69.80 (C-3), 70.21 (C-5), 100.91 (C-1), 127.45 (Ar-CH), 128.4 (Ar-CH), 128.57 (Ar-CH), 129.07 (Ar-CH), 130.17 (Ar-C).

LRMS (ESI^+): 284.2 $[M+H]^+$.

HRMS (ESI) calcd for $C_{14}H_{22}O_5N$ $[M+H]^+$: 284.1492; Found: 284.1489.

6-(4-Dimethoxybenzylamine)-6-deoxy-methyl-α-D-mannopyranoside ***357***

Anisaldehyde (293 mg, 2.16 mmol) was added to a stirred solution of compound **354** (278 mg, 1.44 mmol) in MeOH (20 mL) containing molecular sieves. The reaction mixture was refluxed for 24 hours, filtered and the solvent removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 4 hours. The solution was treated with 2M NaOH (20 mL), filtered and the solvent removed under reduced pressure. The residue was purified by flash chromatography, eluting with DCM/MeOH (5:1) to give the *title compound* **357** (274 mg, 59 %) as a colourless syrup.

$[\alpha]_D^{20}$ = +30 (*c* 0.30, MeOH).

IR: (thin film) 3345, 3004, 2924, 2850, 1677, 1612, 1514, 1455, 1250, 1061, 972 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 2.83 (dd, 1H, *J* = 8.2, 12.7 Hz, H-6), 3.06 (dd, 1H, *J* = 2.8, 12.7 Hz, H-6), 3.36 (s, 3H, OCH_3), 3.46 (t, 1H, *J* = 9.6 Hz, H-4), 3.61-3.65 (m, 2H, H-3, H-5), 3.76 (s, 3H, OCH_3), 3.78 (s, 2H, $ArCH_2$), 3.83 (d, 1H, *J* = 3.8 Hz, H-2), 4.61 (s, 1H, H-1), 6.90 (t, 2H, *J* = 8.2 Hz, Ar-H), 7.29 (t, 2H, *J* = 8.2 Hz, Ar-H).

^{13}C NMR (DMSO, 150.9 MHz): δ 48.44 (C-6), 51.26 (CH_2Ar), 53.51 (OCH_3), 53.59 (OMe), 68.47 (C-4), 69.11 (C-2), 69.79 (C-3), 70.07 (C-5), 100.77 (C-1), 127.58 (Ar-C), 129.32 (Ar-CH), 158.85 (Ar-CH), 158.91 (Ar-C).

LRMS (ESI^+): 314.1 $[M+H]^+$.

HRMS (ESI) calcd for $C_{15}H_{24}O_6N$ $[M+H]^+$: 314.1598; Found: 314.1591.

6-(3,4-Dimethoxybenzylamine)-6-dexoxy-methyl-α-D-mannopyranoside ***358***

3,4-Dimethoxybenzylaldehyde (377 mg, 2.27 mmol) was added to a stirred solution of compound **354** (292 mg, 1.51 mmol) in MeOH (20 mL) containing molecular sieves. The reaction mixture was refluxed for 24 hours, filtered and the solvent removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 4 hours. The solution was treated with 2M NaOH (20 mL), filtered and the solvent removed under reduced pressure. The residue was purified by flash chromatography, eluting with DCM/MeOH (5:1) to give the *title compound* **358** (274 mg, 53%) as a white solid.

$[\alpha]_D^{20}$ = +45.8 (*c* 0.665, MeOH).

IR: (thin film) 3436, 2959, 2927, 2859, 1463, 1272, 1123, 1072, 957 cm^{-1}

^{1}H NMR (D_2O, 600 MHz): δ 2.59 (dd, 1H, *J* = 9.1, 12.4 Hz, H-6), 2.82 (dd, 1H, *J* = 1.9, 12.4 Hz, H-6), 3.23 (s, 3H, OCH_3), 3.35 (t, *J* = 9.6 Hz, H-4), 3.51 (m, 1H, H-5), 3.57 (d, *J* = 3.4 Hz, H-3), 3.59-3.60 (m, 2H, $ArCH_2$), 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.76 (s,1H, H-2), 4.57 (s, 1H, H-1), 6.81 (d, 1H, *J* = 8.2 Hz, Ar-H), 6.86-6.89 (m, 2H, Ar-H).

^{13}C NMR (D_2O, 150.9 MHz): δ 48.80 (C-6), 52.20 (CH_2Ar), 54.80 (OCH_3), 55.66 (OCH_3), 55.75 (OCH_3), 68.89 (C-4), 69.88 (C-2), 70.38 (C-3), 70.66 (C-5), 100.86 (C-1), 111.83 (Ar-CH), 112.29 (Ar-CH) 121.43 (Ar-CH), 132.11 (Ar-C), 147.32 (Ar-C), 148.06 (Ar-C).

LRMS (ESI^+): 344.2 $[M+H]^+$, 366.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{16}H_{26}O_7N$ $[M+H]^+$: 344.1704; Found: 344.1701.

*6-(2-Methoxycyclohexyl)-6-deoxy-methyl-α-D-mannopyranoside **359***

2-Methoxycyclohexanone (280mg, 2.18 mmol) was added to a stirred solution of compound **354** (281 mg, 1.45 mmol) in MeOH (20 mL) containing molecular sieves. The reaction mixture was refluxed for 24 hours, filtered and the solvent removed under reduced pressure. The residue was dissolved in methanol (20 mL); stirred and excess sodium borohydride was added in portions over 4 hours. The solution was treated with 2M NaOH (20 mL), filtered and the solvent removed under reduced pressure. The residue was purified by flash chromatography, eluting with DCM/MeOH (10:1) to give the *title compound* **359** (274 mg, 49 %) as a white solid.

$[\alpha]_D^{20} = +20.3$ (*c* 0.29, $CHCl_3$).

IR: (KBr) 3468, 2957, 2917, 2850, 1576, 1464, 1261, 1096, 871 cm^{-1}.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.08 (m, 2H, Cy), 1.21 (m, 2H, Cy), 1.69 (m, 1H, Cy), 1.76 (m, 1H, Cy), 2.08 (d, 1H, *J* = 1.3 Hz, Cy), 2.16 (m, 1H, Cy), 2.38 (dt, 1H, *J* = 10.3, 4.0 Hz, Cy), 2.92 (m, 2H,), 2.94 (dd, 1H, J = 4.1, 11.5 Hz, H-6), 305 (dd, 1H, *J* = 4.0, 11.5 Hz, H-6), 3.34 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 3.55 (m, 1H, H-4), 3.78 (m, 2H, H-3, H-5), 3.91 (s, 1H, H-2), 4.69 (s, 1H, H-1).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 24.21 (cy), 24.26 (cy), 29.12 (cy), 29.96 (cy), 61.80 (cy), 77.32 (cy), 49.51 (C-6), 55.15 (OCH_3), 56.20 (OCH_3), 68.65 (C-4), 70.17 (C-2), 71.65 (C-3), 72.32 (C-5), 100.96 (C-1).

LRMS (ESI^+): 306.1 $[M+H]^+$.

HRMS (ESI) calcd for $C_{14}H_{28}O_6N$ $[M+H]^+$: 306.1911; Found: 306.1909.

6-*O*-Toluenesulfonyl-methyl-α-D-glucopyranoside **395**

Compound **394** (12.1 g, 62.4 mmol) was dissolved in pyridine (100 mL) and cooled to 0 ^{o}C followed by portionwise addition of TsCl (18.0 g, 94.4 mmol. The reaction mixture was stirred at 0 ^{o}C for 8 hours. The solvent was removed under reduced pressure and

residue was purified by flash chromatography, eluting with DCM/MeOH (25:1) to give the title compound **395** (14.9 g, 69 %) as a colourless syrup.

$[\alpha]_D^{20}$ = +82.5 (*c* 6.66, MeOH).

IR: (thin film) 3393, 3026, 2930, 1598, 1450, 1360, 1176, 1147, 1097, 1057, 972 cm^{-1}.

^1H NMR ($CDCl_3$, 600 MHz): 1.25 (s, 3H, CH_3), 2.16 (s, 3H, OCH_3), 2.24-2.31 (m, 2H, H-2, H-3), 2.52-2.56 (m, 2H, H-5, H-6), 2.71 (s, 1H, H-6), 3.06-3.15 (m, 1H, H-4), 3.49 (d, 1H, *J* = 3.8 Hz, H-1), 6.16 (d, 2H, *J* = 8.1 Hz, Ar H), 6.62 (d, 2H, *J* = 8.3 Hz, Ar H).

^{13}C NMR ($CDCl_3$, 150.9 MHz): δ 21.74 (CH_3), 55.47 (OCH_3) 69.48 (C-6), 69.29 (C-4), 69.64 (C-2), 71.86 (C-3), 74.20 (C-5), 99.43 (C-1), 128.09 (Ar-CH), 129.96 (Ar-CH), 132.81 (Ar-C), 145.05 (Ar-C).

LRMS (ESI^+): 371.2 $[M+Na]^+$.

6-Azido-6-dexoxy-methyl-α-D-glucopyranoside **396**

Sodium azide (8.6 g, 94.6 mmol) was added portionwise to a stirred solution of compound **395** (13.1 g, 33.7 mmol) in DMF (100 mL). The reaction mixture was stirred at 90 ^oC for 5 hours. The solution was filtered to remove white solid. The

solvent was removed under reduced pressure. The crude product was purified by flash chromatography, eluting with DCM/MeOH (15:1) to give the title compound **396** (6.53 g, 79%) as a colourless syrup.

$[\alpha]_D^{20} = +121.3$ (*c* 13.3, $CHCl_3$).

IR: (thin film) 3376, 2928, 2103, 1443, 1353, 1285, 1192, 1147, 1102, 1049, 898 cm^{-1}.

^1H NMR (MeOD, 600 MHz): δ 3.23 (t, 1H, *J* = 9.6 Hz, H-4), 3.32 (s, 3H, OCH_3), 3.37 (dd, 1H, *J* = 3.4, 16.5 Hz, H-6), 3.39 (dd, 1H, *J* = 4.9, 13.2 Hz, H-6), 3.47 (dd, 1H, *J* = 2.2, 13.2 Hz, H-2), 3.65 (dt, 1H, *J* = 9.6, 1.9 Hz, H-5), 4.67 (d, 1H, *J* = 9.2 Hz, H-1).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.40 (C-6), 54.43 (OCH_3), 71.22 (C-4), 71.31 (C-2), 72.08 (C-3), 73.53 (C-5), 99.96 (C-1).

LRMS (ESI^+): 242.1 $[M+Na]^+$.

4-Carbomethoxy-1-(methyl-α-D-mannopyranoside)-1H-[1,2,3]triazole **398**

Compound **353** (237 mg, 1.08 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), methyl propiolate (91 mg, 1.08 mmol) was added followed by

sodium ascorbate (0.108 mmol, 108 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.0108 mmol, 2.70 mg in 100 µL of water). The reaction mixture was heated at 80 ^{o}C for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (25:1) to give the title compound **398** (229 mg, 70%) as a yellow oil.

$[\alpha]_D^{20}$ = +63.3 (*c* 2.19, MeOH).

IR: (thin film) 3406, 3008, 2932, 2839, 1730, 1543, 1375, 1231, 1059, 972 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 3.05 (s, 3H, OCH_3), 3.50 (t, 1H, *J* = 9.6 Hz, H-3), 3.64 (dd, 1H, *J* = 3.4, 18.7 Hz, H-4), 3.76 (dd, 1H, *J* = 1.7, 6.7 Hz, H-2), 3.80 (dt, 1H, *J* = 18.4, 2.4, Hz, H-5), 3.89 (s, 3H, CO_2Me), 4.57 (s, 1H, H-1), 4.59 (dd, 1H, *J* = 8.59, 14.3 Hz, H-6), 4.89 (dd, 1H, *J* = 2.4, 14.3 Hz, H-6), 8.54 (s, 1H, triazol-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.2 (C-6), 51.4 (OCH_3), 53.8 (OMe), 68.2 (C-4), 70.4 (C-2), 70.9 (C-3), 71.1 (C-5), 101.5 (C-1), 129.7, 161.1 (triazole), 138.8 (CO_2Me).

LRMS (ESI^+): 304.2 $[M+H]^+$, 326.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{11}H_{21}O_7N_4$: 321.1405; Found: 321.1404.

4-Hydroxylmethyl-1-(methyl-α-D-mannopyranoside)-1H-[1,2,3]triazole **401**

Compound **353** (322 mg, 1.47 mmol) was dissolved in a 1:1 mixture of water and tert-butyl alcohol (12 mL), propargyl alcohol (83 mg, 1.47 mmol) was added, followed by sodium ascorbate (0.147mmol, 147 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.0147 mmol, 3.7 mg in 100 µL of water). The reaction mixture was stirred at room temperature for 40 hours. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography, eluting with DCM/MeOH (20:1) to give the title compound **401** (222 mg, 58%) as an off-white solid.

mp: 176-177 ^{o}C.

$[\alpha]_D^{20}$ = +7.3 (*c* 8.3, MeOH).

IR: (KBr) 3288, 2957, 2938, 2866, 2836, 1415, 1227, 1137, 1076, 967 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 3.061 (s, 3H, OCH_3) 3.49 (t, 1H, *J* = 9.7 Hz, H-4), 3.64 (dd, 1H, *J* = 3.4, 18.8 Hz, H-3), 3.75 (dd, 1H, *J* = 1.7, 6.3 Hz, H-2), 3.78 (dd, 1H, *J* = 2.2, 18.4 Hz, H-5), 4.49 (dd, 1H, *J* = 8.8, 14.1 Hz, H-6), 4.56 (d, 1H, *J* = 1.3 Hz, H-1), 4.64 (s, 2H, CH_2OH), 4.83 (dd, 1H, *J* = 2.2, 14.1 H-6), 7.94 (s, 1H, triazole-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.21 (C-6), 53.85 (OCH_3), 55.13 ($\underline{C}H_2OH$), 68.33 (C-4), 70.54 (C-2), 71.03 (C-3), 71.48 (C-5), 101.48 (C-1), 124.23, 147.60 (triazole)

LRMS (ESI^+): 276.0 $[M+H]^+$, 298.1 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{10}H_{18}O_6N_3$ $[M+H]^+$: 276.1190; Found: 276.1192.

4-Phenyl1-(methyl-α-D-mannopyranoside)-1H-[1,2,3]triazole **402**

Compound **353** (263 mg, 1.2 mmol) was dissolved in a 1:1 mixture of water and tert-butyl alcohol (12 mL), phenyl acetylene (123 mg, 1.2 mmol) was added followed by sodium ascorbate (0.12 mmol, 120 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.012 mmol, 3.0 mg in 100 µL of water). The reaction mixture was heated at 80 ^oC for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (40:1) to give the title compound **402** (120 mg, 45%) as a colourless oil.

$[\alpha]_D^{20}$ = +19.6 (*c* 0.26, MeOH).

IR: (thin film) 3366, 2930, 2837, 1464, 1444, 1229, 1199, 1132, 1057, 960 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 3.05 (s, 3H, OCH_3), 3.57 (t, 1H, *J* = 3.3 Hz, H-4), 3.68 (dd, 1H, *J* = 3.3, 18.7 Hz, H-3), 3.78 (dd, 1H, *J* = 1.7, 6.7 Hz, H-2), 3.84 (m, 1H, H-5), 4.56 (dd, 1H, *J* = 8.76, 14.3 Hz, H-6), 4.60 (d, 1H, *J* = 1.2, H-1), 4.89 (dd, 1H, *J* = 2.2, 14.3 Hz, H-6), 7.31 (t, 1H, *J* = 1.2 Hz, Ar-H), 7.39 (t, 2H, *J* = 7.9 Hz, Ar-H), 7.78 (d, 2H, *J* = 7.2 Hz, Ar-H), 8.34 (s, 1H, triazole-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.4 (C-6), 53.8 (OCH_3), 68.4 (C-4), 70.6 (C-2), 71.1 (C-3), 71.5 (C-5), 101.5 (C-1), 122.4, 147.3 (triazole), 122.4 (Ar-CH), 128.0 (Ar-CH), 130.40 (Ar-C), 147.28 (Ar-C).

LRMS (ESI^+): 322.2 $[M+H]^+$, 344.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{15}H_{20}O_5N_3$: 322.1397; Found: 322.1403.

4-Butyl-1-(methyl-α-D-mannopyranoside)-1H-[1,2,3]triazole **403**

Compound **353** (207 mg, 0.95 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), 1-hexyne (78 mg, 0.95 mmol) was added followed by sodium ascorbate (0.095 mmol, 95 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.01 mmol, 2.40 mg in 100 µL of water). The reaction mixture was heated at 80 ^{o}C for 15 hours. The solvent was removed under reduced pressure and

the residue was purified by flash chromatography, eluting with DCM/MeOH (20:1) to give the title compound **403** (230 mg, 76%) as white crystals

mp: 138-139 ^{o}C.

$[\alpha]_D^{20} = +71.9$ (*c* 2.05, MeOH).

IR: (KBr) 3367, 3166, 2868, 1553, 1446, 1366, 1311, 1196, 1088 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ 0.921 (t, 3H, *J* = 6.7 Hz, -CH_3), 1.33 (sextet, 2H, *J* = 7.4 Hz, -CH_2-), 1.61 (m, 2H, -CH_2-), 2.67 (t, 2H, *J* = 7.6 Hz, -CH_2-), 3.03 (s, 3H, OCH_3), 3.49 (t, 1H, *J* = 9.6 Hz, H-4), 3.63 (dd, 1H, *J* = 3.4, 18.7 Hz, H-3), 3.73 (dd, 1H, *J* = 2.2, 18.5 Hz, H-5), 3.75 (dd, 1H, *J* = 1.5, 6.7 Hz, H-2), 4.43 (dd, 1H, *J* = 8.76, 14.1 Hz, H-6), 4.55 (d, 1H, *J* = 1.5 Hz, H-1), 4.80 (dd, 1H, *J* = 2.2, 14.1 Hz, H-6), 7.76 (s, 1H, triazole-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 12.8 (C-4'), 21.8 (C-3'), 24.6 (C-2'), 31.5 (C-1'), 51.16 (C-6), 53.68 (OCH_3), 68.4 (C-4), 70.5 (C-2), 71.1 (C-3), 71.6 (C-5), 101.5 (C-1), 123.2, 147.6 (triazole).

LRMS (ESI^{+}): 302.1 $[M+H]^{+}$, 324.2 $[M+Na]^{+}$.

HRMS (ESI) calcd for $C_{13}H_{24}O_5N_3$: 302.1710; Found: 302.1705.

4-Cyclohexyl-1-(methyl-α-D-mannopyranoside)-1H-[1,2,3]triazole **404**

Compound **353** (228 mg, 1.04 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), cyclohexyl acetylene (113 mg, 1.04 mmol) was added, followed by sodium ascorbate (0.104 mmol, 104 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.0104 mmol, 2.60 mg in 100 µL of water). The reaction mixture was heated at 80 ^{o}C for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography eluting with DCM/MeOH (20:1) to give the title compound **404** (192 mg, 56%) as white crystals.

mp: 119-121 ^{o}C.

$[\alpha]_D^{20}$ = +33.2 (*c* 0.545, MeOH).

IR: (KBr) 3393, 2928, 2852, 1450, 1381, 1246, 1131, 1059, 969 cm^{-1}

^{1}H NMR (MeOD, 600 MHz): δ 1.27 (m 1H, cy), 1.40 (q, 4H, cy), 1.71 (d, 1H, *J* = 12.8 Hz, cy), 1.78 (m, 2H, cy), 1.96 (m, 2H, cy), 2.70 (m, 1H, cy), 3.02 (s, 3H, OCH_3), 3.51 (t, 1H, *J* = 9.4 Hz, H-4), 3.64 (dd, 1H, *J* = 3.3, 18.6 Hz, H-3), 3.73 (dt, 1H, *J* = 11.4, 9.5

Hz, H-5), 3.76 (dd, 1H, *J* = 1.7, 6.5 Hz, H-2), 4.43 (dd, 1H, *J* = 8.9, 14.1 Hz, H-6), 4.56 (d, 1H, 1.2 Hz, H-1), 4.82 (dd, 1H, *J* = 2.06, 14.1 Hz, H-6), 7.75 (s, 1H, triazol-H). ^{13}C NMR (MeOD, 150.9 MHz): δ 25.78 (C-4'), 25.91 (C-3'), 32.87 (C-2'), 35.14 (C-1'), 51.21 (C-6), 53.74 (OCH_3), 68.42 (C-4), 70.54 (C-2), 71.10 (C-3), 71.66 (C-5), 101.44 (C-1), 121.93, 152.98 (triazole).

LRMS (ESI^+): 328.3 $[M+H]^+$, 350.3 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{15}H_{26}O_5N_3$: 328.1867; Found: 328.1866.

4-Carbomethoxy-1-(methyl-α-D-glucopyranoside)-1H-[1,2,3]triazole **405**

Compound **396** (239 mg, 1.09 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), methyl propiolate (92 mg, 1.09 mmol) was added, followed by sodium ascorbate (0.109 mmol, 109 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.011 mmol, 2.72 mg in 100 µL of water). The reaction mixture was heated at 80 ^oC for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (20:1) to give the title compound **405** (229 mg, 69%) as a white solid

mp: 155-157 ^oC.

$[\alpha]_D^{20}$ = +100.6 (*c* 0.79, MeOH).

V_{max} (KBr): 3433, 3121, 3003, 2935, 2901, 1725, 1438, 1350, 1236, 1144, 1072 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ 3.11 (s, 3H, OCH_3), 3.29 (quintet, 1H, *J* = 1.6 Hz, H-4), 3.33 (dd, 1H, *J* = 3.8, 17.2 Hz, H-2), 3.59 (t, 1H, *J* = 9.3 Hz, H-3), (dt, 1H, *J* = 7.2, 9.2 Hz, H-5), 3.89 (s, 3H, OMe), 4.59 (dd, 1H, *J* = 8.08, 14.1 Hz, H-6), 4.61 (d, 1H, *J* = 3.8 Hz, H-1), 4.85 (d, 1H, *J* = 2.58 Hz, H-6), 8.51 (s, 1H, triazole-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.24 (C-6), 51.37 (OCH_3), 54.21 (OMe), 70.08 (C-4), 71.62 (C-2), 71.69 (C-3), 73.53 (C-5), 99.99 (C-1), 129.74, 161.09 (triazole), 138.93 (C=O).

LRMS (ESI^+): 304.1 $[M+H]^+$, 326.2 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{11}H_{21}O_7N_4$: 321.1405; Found: 321.1405.

4-Hydroxymethyl-1-(methyl-α-D-glucopyranoside)-1H-[1,2,3]triazole **406**

Compound **396** (271 mg, 1.24 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), propargyl alcohol (63 mg, 1.24 mmol) was added followed by sodium ascorbate (0.124 mmol, 139 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.0124 mmol, 3.1 mg in 100 µL of water). The

reaction mixture was heated at 80 ^{o}C for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (40:1) to give the title compound **406** (242 mg, 85%) as an off-white solid.

mp: 220-223 ^{o}C.

$[\alpha]_D^{20}$ = +22.4 (*c* 1.65, MeOH).

IR(KBr): 3371, 3296, 2915, 2839, 1363, 1229, 1196, 1151, 1092 cm^{-1}.

^{1}H NMR (D_2O, 600 MHz): δ 2.97 (s, 3H, OCH_3), 3.07 (t, 1H, *J* = 9.1 Hz, H-4), 3.36 (dd, 1H, *J* = 3.7, 19.6 Hz, H-2), 3.50 (t, 1H, *J* = 9.6 Hz, H-3), (dt, 1H, *J* = 12.0, 10.1 Hz, H-5), 4.48 (dd, 1H, *J* = 8.1, 14.8 Hz, H-6), 4.56 (s, 2H, CH_2OH) 4.57 (d, 1H, *J* = 3.8 Hz, H-1), 4.69 (m, 1H, H-6), 7.88 (s, 1H, triazole-H).

^{13}C NMR (D_2O, 150.9 MHz): δ 50.85 (C-6), 54.55 (OCH_3), 54.78 (CH_2OH), 69.9 (C-4), 70.83 (C-2), 71.06(C-3), 73.02 (C-5), 99.15 (C-1), 125.14, 146.82 (triazole).

LRMS (ESI^+): 276.1 $[M+H]^+$, 298.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{10}H_{18}O_6N_3$ $[M+H]^+$: 276.1190; Found: 276.1191.

4-Phenyl1-(methyl-α-D-glucopyranoside)-1H-[1,2,3]triazole **407**

Compound **396** (305 mg, 1.39 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), phenyl acetylene (142 mg, 1.39 mmol) was added, followed by sodium ascorbate (0.139 mmol, 139 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.014 mmol, 3.5 mg in 100 µL of water). The reaction mixture was heated at 80 ^{o}C for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (40:1) to give the title compound **407** (151 mg, 34%) as a white solid.

mp: 206-207 ^{o}C.

$[\alpha]_D^{20}$ = +90.2 (*c* 1.48, $CHCl_3$).

IR (KBr): 3397, 3336, 2924, 2891, 2840, 1608, 1466, 1375, 1223, 1155, 1047 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ 3.13 (s, 3H, OCH_3), 3.15 (m, 1H, H-4), 3.36 (dd, 1H, *J* = 3.8, 19.5 Hz, H-2), 3.61 (t, 1H, *J* = 9.3 Hz, H-3), 3.89 (dt, 1H, *J* = 10.2, 8.3 Hz, H-5), 4.55 (dd, 1H, *J* = 8.1, 14.3 Hz, H-6), 4.64 (d, 1H, *J* = 3.6 Hz, H-1), 4.84 (d, 1H, *J* = 2.4 Hz, H-6), 7.32 (t, 1H, *J* = 7.5 Hz, Ar H), 7.41 (t, 2H, *J* = 7.7 Hz, Ar H), 7.79 (d, 2H, *J* = 7.8 Hz, Ar H), 8.32 (s, 1H, triazole H).

^{13}C NMR (MeOD, 150.9 MHz): δ 51.20 (C-6), 54.18 (OCH_3), 70.41 (C-4), 71.55 (C-2), 71.68 (C-3), 73.63 (C-5), 99.95 (C-1), 122.29, 147.30 (triazole), 122.29 (Ar-CH), 125.29 (Ar-CH), 128.0 (Ar-CH), 128.66 (Ar-CH), 130.36 (Ar-C),

LRMS (ESI^+): 344.0 $[M+Na]^+$.

HRMS (ESI) calcd for $C_{15}H_{20}O_5N_3$: 322.1397; Found: 322.1396.

4-Cyclohexyl-1-(methyl-α-D-glucopyranoside)-1H-[1,2,3]triazole **408**

Compound **396** (307 mg, 1.40 mmol) was dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL), cyclohexyl acetylene (152 mg, 1.40 mmol) was added followed by sodium ascorbate (0.140 mmol, 140 µL of a freshly prepared 1M solution in water) and copper(II) sulphate pentahydrate (0.0140 mmol, 3.5 mg in 100 µL of water). The reaction mixture was heated at 80 ^oC for 15 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with DCM/MeOH (30:1) to give the title compound **408** (305 mg, 67%) as a white solid.

mp: 157-159 ^oC.

IR: (KBr) 3362, 2925, 2852, 1548, 1450, 1364, 1195, 1151, 1049 cm^{-1}.

^{1}H NMR (MeOD, 600 MHz): δ 1.27 (m, 1H, cy), 1.42 (m, 4H, cy), 1.72 (m, 1H, cy), 1.79 (m, 2H, cy), 1.98 (m, 2H, cy), 2.70 (m, 1H, CHN), 3.09 (s, 3H, OCH_3), 3.12 (m, 1H, H-4), 3.35 (dd, 1H, *J* = 3.7, 19.3 Hz, H-2), 3.59 (t, 1H, *J* = 3.8 Hz, H-3), 3.79 (dt, 1H, *J* = 10.3, 8.4 Hz, H-5), 4.43 (dd, 1H, *J* = 8.4, 15.1 Hz, H-6), 4.60 (d, 1H, *J* = 3.8 Hz, H-1), (dd, 1H, *J* = 2.3, 15.1 Hz, H-6), 7.72 (s, 1H, triazole-H).

^{13}C NMR (MeOD, 150.9 MHz): δ 25.63 (C-4'), 25.76 (C-3'), 32.73 (C-2'), 35.0 (C-1'), 50.94 (C-6), 53.99 (OCH_3), 70.43 (C-4), 71.54 (C-2), 71.89 (C-3), 73.49(C-5), 99.72 (C-1), 121.75, 152.89 (triazole).

LRMS (ESI^+): 328.1 $[M+H]^+$.

HRMS (ESI) calcd for $C_{15}H_{26}O_5N_3$: 328.1867; Found: 328.1864.

Biological Assay

Determination of the Inhibition of Jack bean α-mannosidase:[162]

Materials:

The **NaOAc Buffer** (pH = 4.5, containing 2 mM $ZnCl_2$) was prepared by combining 150 mL of a 0.2 M solution of AcOH (made by dissolving 5.72 mL in 500 mL water), 100 mL of a 0.2 M solution of NaOAc (made by dissolving 8.20 g in 500 mL water) and $ZnCl_2$ (273 mg) and making up the resulting solution to 1000 mL with water. The pH was adjusted to 4.5, if necessary, by adding either NaOAc or AcOH solution.

The **borate buffer** (pH = 9.0) was prepared by mixing 100 mL of a 0.02 M solution of boric acid (made by dissolving 1.24 g of H_3BO_4 in 100 mL of H_2O) with 400 mL of a

0.005 M solution of sodium tetraborate decahydrate (made by dissolving 9.53g in 500 mL of H_2O).

The **enzyme stock solution** ([E] = 95 mU/mL) was prepared by diluting 5 μL of Jack bean α-mannosidase (Sigma M-7257, 95 U/mL, supplied as a suspension in 3.0 M $(NH_4)_2SO_4$ and 0.1 mM $Zn(OAc)_2$), with 4995 μL NaOAc-buffer (pH = 4.5, containing 2 mM Zn Cl_2).

The **substrate stock solution** ([S] = 20.0 mM) was prepared by dissolving *p*-nitrophenyl-α-D-mannopyranoside (18.4 mg, 61 mmol) in 3050 μL of NaOAc buffer (pH = 4.5, containing 2 mM $ZnCl_2$).

Procedure:

The inhibition tests were performed by incubating 50 μL of enzyme solution and 20 μL of inhibitor solution or H_2O for 30 min at 25 ^oC. The reaction was started by addition of 30 μL of substrate solution. The velocity of the substrate hydrolysis was determined by quenching the reaction after 5 min by adding 100 μL of borate buffer and measuring the absorption at 405 nm.

Calculations:

K_M (1.5 mM) was determined by performing the above experiment with six different substrate concentrations (with enzyme and H_2O) and plotting the inverse substrate concentration vs. inverse rate of hydrolysis (Lineweaver Burk plot). The IC_{50} values were determined by performing the above experiment with six different inhibitor concentrations at a substrate concentration corresponding to K_M. Determination of the inhibition constants (K_i) was performed at different concentrations of the inhibitor (usually six) bracketing the K_i or IC_{50} value at six different substrate concentrations bracketing the K_M. IC_{50} values were calculated by plotting the inhibitor concentration vs. the rate of hydrolysis. K_i and α values were determined from the replot of the slopes and the replot of the 1/v axis intercepts of Lineweaver Burk plots

MTT Assay

Cell lines:

Colorectal HCT116 $^{p53+/+}$ (both p53 alleles intact) and HCT116 $^{p53-/-}$ (both p53 alleles disrupted) were used. (Bunz F., Dutriaux A., Lengauer C., Waldman T., Zhou S., Brown J. P., Sedivy J. M., Kinzler K. W., Vogelstein B. *Science*, **1998**, *282, 1497–1501*).

Preparation of test compounds:

Test compound was dissolved in DMSO to make up a 400 mM solution. From this stock solution appropriate aliquots were used to make up a 100μM solution of the test compounds in RPMI 1640 medium (final DMSO content ≤ 0.1%). Similarly, appropriate aliquots were used this from this 100 μM solution to make up a further 9 solutions of the test compounds in RPMI 1640 medium. The solutions were stored at -20 ^{o}C until required.

Procedure:

Cells were plated in 96-well plates with 200 μL of cell suspension (0.5 x 10^4 cell/mL) per well. Cells were left to adhere overnight at 37 ^{o}C in humidified incubator with 5% CO_2. The cells were then treated with a range of concentrations of the test compound for 60 minutes. Each plate also contained a blank group (no test compound). Plates were then incubate for further 96 h (in 5% CO_2 and 95% air humidified incubator at 37 ^{o}C) before 20 μL of MTT solution (5 mg/mL) were added to each well. The plate was then incubated in the dark at 37 ^{o}C for 4 h. The supernatant was removed and formazan crystals were dissolved by addition of DMSO (150 μL). The absorbance was read immediately at 540 nm and % cell survival calculated as follows:

This assay was performed in triplicate. The mean IC_{50} (the concentration of drug that is required for 50% growth inhibition) ± SD was determined.

References:

[1] Suami, T.; Ogawa, S. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 21–90.

[2] Suami, T. *Pure Appl. Chem*. **1987**, *59*, 1509-1520.

[3] (a) de Melo, E. B.; Gomes, A. D.; Carvalho, I. *Teterahedron* **2006**, *62*, 10277. (b) Zou, W. *Curr. Topics Med. Chem.* **2005**, *5*, 1363. (c) Somsak, L.; Nagy, V.; Hadady, Z.; Docsa, T., Gergely, P. *Curr. Pharm. Design* **2003**, *9*, 1177. (d) Vogel, P. *Chimia* **2001**, *55*, 359. (e) Yuasa, H.; Hashimoto, H. *Trends Glycosci. Glycotech.* **2001**, *13*, 31.

[4] Delgado, A. *Eur. J. Org. Chem.* **2008**, 3893–3906.

[5] Parry, R. J.; Bornemann, V. *J. Am. Chem. Soc.* **1985,** *107***,** 6402-6403.

[6] Wrodnigg, T. M.; Steiner, A. J.; Ueberbacher, B. J. *Anti-Cancer Agents In Med. Chem*. **2008**, *8*, 77-75.

[7] Mohamud, T. *Nat. Prod. Rep.* **2003**, *20*, 137-166.

[8] Cipolla, L.; La Ferla, B.; Nicotra, F. *Curr. Topics Med. Chem.* **2003**, 3, 485-511.

[9] L. Vértesy, H-W. Fehlhaber and A. Schulz, *Angew. Chem., Int. Ed*, **1994**, *33*, 1844-1846.

[10] Compain, P.; Martin, O. R. *Curr. Topics Med. Chem*. **2003**, 541-560.

[11] Yuasa, H.; Palcic, M. M.; Hindsgaul, O. *Can. J. Chem.* **1995**, *73*, 2190-2195.

[12] Compain, P.; Martin, P.R. *Bioorg. Med. Chem.* **2001**, 9, 3077-3092

[13] Okada, Y.; Yamada, S.; Toyoshima, M.; Dong, J.; Nakajima, M.; Sugahara, K. *J.Biol. Chem.* **2002** *277*, 42488-42495

[14] Ogawa, S. *Tren in Glycosci Glycotech*. **2004**, *16*, 33-53.

[15] Ogawa, S.; Shibata, Y.; Chida, N.; Ito, H.; Suami, T. *Chem. Lett.* **1980**, 135-138.

[16] Horii, S.; Iwasa, T.; Kameda, Y. *J. Chem. Soc., Chem. Commun.* **1972**, 747.

[17] Ogawa, S.; Shibata, Y.; Chida, N.; Ito, H.; Suami, T. *Bull. Chem.. Soc. Jpn*, **1983**, *56*, 494-498.

[18] Ogawa, S.; Chida, N.; Ito, H.; Suami, T. *Bull. Chem.. Soc. Jpn*, **1983**, *56*, 499-504.

[19] Ogawa, S,; Shibata, Y.; Miyazawa, K.; Toyokuni, T.; Iida, T. *Carbohydr. Res.* **1987**, *163*, 53-62.

[20] Birch, G. *Adv. Carbohydr. Chem.* **1963**, *18*, 201-225.

[21] (a) Elbein, A. Adv. Carbohydr. Chem. Biochem. 1974, *30*, 227-256. (b) Bock, K.; Defaye, I.; Driques, H; Bar-Guilloijx, E. *Eur. I. Biochem.* **1983**, *131*, 595-600.

[22] Ogawa, S,; Yokoi, S,; Kimura, N.; Shibata, Y.; Chida, N. *Carb. Res.* **1988**, *181*, 57-66.

[23] Ogawa, S.; Sugawa, I.; Shibata, Y. *Carbohydr. Res.* **1991**, *211*, 147-155.

[24] Tsunoda, H.; Sasaki, S.; Furuya, T.; Ogawa, S. *Liebigs Ann.* **1996**, 159–165.

[25] Ogawa, S.; Hirai, K.; Ohno, M.; Furuya, T.; Sasaki, S.; Tsunoda, H. *Liesbigs Ann.* **1996**, 673-677.

[26] Okazaki, K.; Nishigaki, S.; Ishizuka, F.; Kajihara, Y.; Ogawa, S. *Org. Biomol. Chem.* **2003**, *1*, 2229-2230.

[27] Ogawa, S.; Hirai, K.; Odagiri, T.; Matsunaga, N.; Yamazaki T.; Nakajima, A. *Eur. J. Org. Chem.* **1998**, 1099–1109.

[28] Schaub, C.; Muller, B.; Schmidt, R. *Eur. J. Org. Chem.*, **2000**, 1745–1758.

[29] Fogel, M.; Altevogt, P.; Schirrmacher, V. *J. Exp. Med.*, **1983**, *157*, 371–376.

[30] Mari, S.; Posteri, H.; Marcou,G.; Potenza, D.; Micheli, F.; Cańada, F. J.; Jimenez-Barbero, J.; Bernardi, A. *Eur. J. Org. Chem.* **2004**, 5119-5125.

[31] Reina, J. J.; Sattin, S.; Invernizzi, D.; Mari, S.; Mart_nez-Prats, L.; Tabarani, G.; Fieschi, F.; Delgado, R.; Nieto, P. M.; Rojo, J.; Bernardi, A. *Chem. Med. Chem.* **2007**, *2*, 1030-1036.

[32] Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Minura, Y.; R. Kunert, Zhu, P.; Wormald, M. R. ; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science*,**2003**, 300, 2065–2071.

[33] Ogawa, S.; Sasaki, S.; Tsunoda, H. *Chem. Lett.* **1993**, 1587-1590.

[34] Paulsen, H.; Deyn, W. V. *Liebigs Ann.* **1987**, 141–152.

[35] Ogawa, S.; Furuya, T.; Tsunoda, H.; Hindsgaul, O.; Stangier, K.; Palcic, M. M. *Carb. Res.* **1995**, *271*, 197-205.

[36] Ogawa, S.; Sasaki, S.; Tsunoda, H. *Carb. Res.* **1995**, *274*, 183-196.

[37] S. K. Das, J. M. Mallet, P. Sinaÿ, *Angew. Chem. Int. Ed.* **1997**, *36*, 493-496.

[38] R. J. Ferrier, *J. Chem. Soc., Perkin Trans. 1* **1979**, 1 1455-1458. (b) R. J. Ferrier, S. Middleton, *Chem. Rev.* **1993**, 93, 2779-2831.

[39] Pearce, A. J.; Sollogoub, M.; Mallet, J.-M.; Sinaÿ, P. *Eur. J. Org. Chem.* **1999**, 2103-2117.

[40] Sollogoub, M.; Pearce, A. J.; Hérault, A.; Sinaÿ, P. *Tetrahedron: Asymmetry* **2000**, *11*, 283-294.

[41] Pearce, A. J.; Sollogoub, M.; Mallet, J.-M.; Sinaÿ, P. *Tetrahedron Lett.* **1998**, *39*, 3471-3472.

[42] Koto, S.; Morishima, N.; Kawahara, R.; Ishikawa, K.; Zen, S. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1092-1096.

[43] Limori, T.; Takahashi, H,; Ikegami, S. *Tetrahedron Lett.* **1996**, *37*, 649-652.

[44] Cheng, X.; Khan, N.; Kumaran, G.; Mootoo, D. R. *Org. Lett*. **2001**, *3*, 1323-1325.

[45] (a) Cheng, X.; Khan, N.; Mootoo, D. R. *J. Org. Chem*. **2000**, *65*, 2544-2547. (b) Khan, N.; Cheng, X.; Mootoo, D. R. *J. Am. Chem. Soc*. **1999**, *121*, 4918-4919.

[46] (a) Keck, G. E.; Kachensky, D. F.; Enholm, E. J. *J. Org. Chem*. **1985**, *50*, 4317-4325. (b) Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R.*Tetrahedron* **1985**, *41*, 4079-4094.

[47] Larsen, D. S.; Trotter, N. S.; Stoodley, R. J. *Tetrahedron Lett*. **1993**, 34, 8151-8154.

[48] Larsen, D. S.; Trotter, N. S.; Stoodley, R. J. Brooker, S. *Tetrahedron Lett*. **2000**, 41, 8957-8962.

[49] Larsen, D. S.; Roger, J. L.; Trotter, N. S.; Stoodley, R. J.*J. Chem. Soc., Perkin Trans. 1*, **2001**,

2204–2212.

[50] Larsen, D. S.; Roger, J. L.; Trotter, N. S.; Stoodley, R. J. *Org. Biomol. Chem.* **2004**, *2*, 1934-1942.

[51] (a) Barton, D. H. R.; Zard, S. Z. *Pure Appl. Chem.*, **1986**, 58, 675. (b) Barton, D. H. R.; Zard, S. Z.; Bridon, D. *Chem. Commun.*, **1985**, 1066-1068.

[52] Pritchard, R. G.; Stoodley, R. J.; Yuen, W.-H. *Org. Biomol. Chem.* **2005**, *3*, 162-171.

[53] Alberecht, W. *Ann*. **1906**, *348*, 31.

[54] Diels, O.; Alder, K. *Ann*. **1928**, *460*, 98-122.

[55] Fleming, I.; *Frontier Orbitals and Organic Chemical Reactions*, Wiley, Chichester, **1978**, 87-181.

[56] Afarinkia, K.; Bearpark, M. J.; Ndibmawi, A. *J. Org. Chem.* **2003**, *68*, 7158-7166.

[57] Shusherina, N. P.; Pilipenko, V. S.; Kireeva, 0. K.; Geller, B. I.; Stepanyants, A. U. J. *Org. Chem. USSR* **1980**,*16*, 2047-2051.

[58] Afarinkia, K.; Ndibmawi, A. *Synlett.* **2007**, 1940-1943.

[59] Schmidt O.T.; Whistler, R.L.; Wolfram, M.L. *Methods in Carbohydrate Chemistry*, vol. II, Academic Press Inc., New York, 1963, 321-322.

[60] Handerson, S.; Schalf, M. *Org. Lett.* **2002**, *4*, 407-409.

[61] Rollin, P.; Verez Bencomo, V.; Sinay, P. *Synthesis* **1984**, 134-135.

[62] Perrine, T. D.; Glaudemans, R. K.; Ness, R. K.; Kyle, J.; Fletcher, H. G., Jr. *J. Org. Chem.* **1967**, *32*, 664-669.

[63] Cottier, L.; Remy, G.; Descotes, G. *Synthesis* **1979**, 711-712.

[64] Black, W. A. P.; Dewar, E. T.; Rutherford, D. *J. Chem. Soc.* **1963**, 4433-4439.

[65] Edathil, J.; Nguyen, J.; Hughes, K.; Boyko, W. J.; Giuliano, R. M. *J. Carbohydr. Chem.* **2001**, *20*, 81-85.

[66] Reynolds, D. D.; Evans, W. .; *Org. synth.* **1942**, *22*, 56**.**

[67] Brimble, M. A., Kowalczyk, R., Harris, P. W. R., Dunbar, P. R., Muir, V. J. *Org. Biomol. Chem.*, **2008**, *6*, 112-121.

[68] Posner, G. H.; Afarinkia, K.; Dai, H.; *Org. synth.* **1998**, *9*, 112.

[69] Afarinkia, K.; Mohmood, F. *Tetrahedron*, **1999**, *55*, 3129-3140.

[70] Posner, G. H.; Afarinkia, K. *Tetrahedron Lett*. **1992**, *53*, 7839-7842.

[71] Afarinkia, K.; Nelson, T. D.; Vinader, M. V.; Posner, G. H. *Tetrahedron* **1992**, *48*, 9111–9171.

[72] Afarinkia, K.; Bahar, A.; Neuss, J. *Synlett* **2003**, 2341–2344.

[73] Afarinkia, K.; Bearpark, M. J.; Ndibmawi, A. *J. Org. Chem.* **2005**, *70*, 1122-1133.

[74] Posner, G. H.; Johnson, N. *JOC*, **1994**, *59*, 7855-7861. Posner, G. H.; Cho, C.-G.; Anjeh, T. E. N.; Johnson, N.; Horst, J. R. L.; Kobayashi, T.; Okano, T.; Tsugawag, N. *J. Org. Chem.*, **1995**, *60*, 4619-4628.

[75] Freudenberg, K.; Dürr, W.; Hochstetter, H. V. *Chem. Ber*. **1928**, *61*, 1735-1743.

[76] Bird, C. W. *Tetrahedron*, **1986**, *42*, 89-92.

[77] Woodward, B. T.; Posner, G. H. *Advances in Cyloaddition*, **1999**, *5*, 47.

[78] (a) Shimo, T.; Kataoka, K.; Maeda, A.; Somekawa, K. *J. Heterocyclic Chem.* **1992**, 811-813. (b) Passarella, D.; Lesma, G.; Martinelli, M.; Silvani, A.; Canto, M.; Hidalgo, J. *Tetrahedron* **2000**, *56*, 5205-5208. (c) Matsushita, Y.; Sakamoto, K.; Murakami, T.; Matsui, T. *Synth. Commun*. **1994**, 24, 3307-3314. (d) Murakami, N.; Tanase, T.; Nagai, S.; Sato, Y.; Ueda, T.; Sakakibara, J.; Ando, H.; Hotta, Y.; Takeya, K. *Chem. Pharm. Bull.* **1991**, *39*, 1962-1966.

[79] (a) Cantin, A.; Corma, A.; Diaz-Cabanas, M. J.; Jorda, J. L.; Moliner, M. *J. Am. Chem. Soc.* **2006**, *128*; 4216-4217. (b) Shimo, T.; Kataoka, K.; Maeda, A.; Somekawa, K. *J. Heterocyclic Chem.* **1992**, *29*, 811-813. (c) Zimmerman, H. E.; Paufler, R. M. *J. Am. Chem. Soc.* **1960**, *82*, 1514-1415.

[80] (a) Ciganek, E. *J. Am. Chem. Soc.* **1981**, *103*, 6262-6263. (b) Boger, D. L.; Takahashi. K. *J. Am. Chem. Soc.* **1995**, *117*, 12452-12459.

[81] Markó, I. E.; Evans, G. R.; Declercq, J.-P. *Tetrahedron*, **1994**, *50*, 4557-4574.

[82] Gaussian Development Version, Revision H.08, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian,A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. *We thank Dr Michael Bearpark for carrying out the calculations.*

[83] Dewar, M. J. S., Zoebisch, E. G.; Healy, E. F.; Stewart J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902; Dewar, M. J. S., Zoebisch, E. G. *THEOCHEM (J. Mol. Structure)* **1988**, *180*, 1- 21.

[84] Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648-5652.

[85] Trost, B. M.; Chupak, L. S.; Lübbers, T. *J. Am. Chem. Soc.* **1998,** *120*, 1732-1740.

[86] Bromfield, K. M.; Gradén, H.; Hagberg, D. P.; Olssonb, T.; Kann, N. *Chem. Commun*. **2007**, 3183–3185.

[87] Brettle, R.; Cross, R.; Frederickson, M.; Haslam, E.; Davies G. M. *Bioorg & Med. Chem. Lett.* **1996**, *6*, 291-294.

[88] Gano, K. W.; Monbouquette, H. G.; Myles, D. C. *Tetrahedron Lett*., **2001**, *42*, 2249–2251.

[89] Mera, S. M. Pathology & understanding Disease Prevention, Stanley Thornes, p379-429.

[90] Lederberg, J. Infectious History. www.univie.ac.at.

[91] Sohami, R. L.; Moxham, J. Medicine, 3rd Edition, p133-154.

[92] Sherr, C. J. *Science*, **1996**, *274*, 1672-1677.

[93] Weinberg, R. A. *Scientific American*. **1996**, 62-70.

[94] Jacobson, M. D.; Weil, M.; Raff, M. *C. Cell*, **1997**, *88*, 347-354.

[95] Dominiczak, B. Medical Biochemistry, Mosby.

[96] Andreeff, M.; Goodrich, D. W.; Pardee, A. Cell Proliferation, Differentiation and Apoptosis. www.cancer.org.

[97] Metzler, D. E. *Biochemistry the Chemical Reactions of Living Cells*, Vol. 2, 2nd Edition.

[98] Bertram, J. S. *Mol. Asp. Mel. Bio. Canc*. **2001**, *21*, 167-223.

[99] Donnelly, J. G. *The drug Monitor*. **2004**, *26*, 231-235.

[100] Fiax, H.; Popowycs, F.; Favre, S.; Schutz, C.; Vogel, P.; Gerber-Lemaire, S.; Juillerat-Jeanneret, L. *J. Med Chem*. **2005**, *48*, 4237-4246.

[101] Picture from http://www.faculty.uca.ed.htm.

[102] Garrett, R. H.; Grisham, C. M. Biochemistry, 2nd Eddition, p284-288.

[103] Verbert, A.; Cacan, R. *Encyclopedia of life science*, **2001**, nature publishing Group.

[104] Murray, R. K. *GlycoScience & Nutrition*, **2003**, 4, 7, http://www.glycoscience.org.

[105] Moremen, K. W. *Biochim Biophys. Acta*, **2002**, *1573*, 225-235.

[106] Fuhrmann, U.; Bause, E.; Ploegh, H. *Biochim. Biophys. Acta*, **1985**, *825*, 95-110.

[107] Goss, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W. *Clin. Canc. Res*. **1995**, *1*, 935-944.

[108] Elsen, D.; Kuntz, D. A.; Rose, D. R. *EMBO*, **2001**, *20*, 3008- 3017.

[109] Saotome, C.; Wong, C. H.; Kanie, O. *Chem. & Bio*. **2001**, *8*, 1061-1070.

[110] Rye, C. S.; Withers, S. G. *Curr. Opin. Chem. Bio*. **2000**, *4*, 573-580.

[111] Vasella, A. T.; Heightman, T. D. *Angew. Chem. Int. Ed*. **1999**, *38*, 750-770.

[112] Goss, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W. *Clin. Canc. Res*. **1995**, *1*, 935-944.

[113] Pearson, W. H.; Guo, L. *Tetrahedron Lett*. **2001**, *42*, 8267-8271.

[114] Pearson, W. H.; Hembre, J. H. *Tetrahedron Lett*. **2001**, *42*, 8273-8276.

[115] Goss, P. E.; Baptiste, J.; Fernandes, B.; Baker, M.; Dennis, J. W. *Cancer. Res*. **1994**, *15*, 1450-1457.

[116] Desmet, T.; Nerinckx, W.; Stals, I.; Callewaert, N.; Conteras, R.; Claeyssens, *M. Anal. Biochem*. **2002**, *307*, 361-367.

[117] Herscovics, A. *Biochim Biophys. Acta*, **1999**, *1473*, 96-107.

[118] Dennis, J. W.; White, S. L.; Freer, A. M.; Mime, D. *Biochem. Pharm*. **1993**, *46*, 1459-1466.

[119] Olden, K.; Breton, P.; Grzegorzewski, K.; Yasuda, Y.; Gause, B. L.; Oredipe, O. A.; Newton, S. A. *Pharmac. Ther*. **1991**, *50*, 285-290.

[120] Elbein, A. D. *FASEB* **1991**, *5*, 3055-3063.

[121] (a) Gao-Feng, S.; Jia-Qi, L.; Xiao-Ping, J.; Ying, C. *Tetrahedron*, **2008**, *64*, 5005-5012. (b) Ceccon, J.; Andrew, A. E.; Poisson, J.-F. *Org. Lett*. **2006**, *8*, 4739-4742. (c) Au, C. W. G.; Pyne, S. G. *J.Org. Chem*. **2006**, *71*, 7097-7099. (d) Mukai, C.; Sugimoto, Y-I.; Miyazawa, K.; Yamaguchi, S.; Hanaoka, M. *JOC*, **1998**, *63*, 6281-6287. (e) Zhou, W-S.; Xie, W-G.; Lu, Z-H; Pan, X-F. *Org. Biorg. Chem*. **1995**, *20*, 2599-2604.

[122] Zhou, W. S.; Xie, W. G.; Lu, Z. H.; Pan, X. F. *Tetrahedron Lett*. **1995**, *36*, 1291-1294.

[123] Goss, P. E.; Reid, C. L.; Bailey, D.; Dennis, J. W. *Clin. Canc. Res*. **1997**, *3*, 1077-1086.

[124] Popowycz, F.; Gerber-Lemaire, S.; Rodriguez-Garcia, E.; Schutz, C.; Vogel, P. *Helv. Chim. Acta*, **2003**, *86*, 1914-1948.

[125] Hembre, E. J.; Pearson, W. H. *Tetrahedron* **1997**, *53*, 11021-11032.

[126] Li, B.; Kawatkar, S. P.; George, S.; Strachan, H.; Woods, R. J.; Siriwardena, A.; Moremen, K. W.; Boons,G. *Chem. Bio. Chem*. **2004**, *5*, 1220-1227.

[127] Kunts, D. A.; Ahmad, G.; Johnston, B. D.; Pinto, B. M.; Rose, D. R. *Tetrahedron Assym.* **2005**, *16*, 25-32.

[128] Nishimura, Y.; Adachi, H.; Satoh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. *J. Org. Chem*. **2000**, *65*, 4871-4882.

[129] Terinek, M.; Vasella, A. *Helv. Chim. Acta*, **2003**, *86*, 3482-3509.

[130] Behr, J. B. ; Chevrier, C. ; Defoin, A. ; Tarnus, C.; Streith, J. *Tetrahedron* **2003**, *59*, 543-553.

[131] Kavlekar, L. M.; Kuntz, D. A.; Wen, X.; Johnston, B. D.; Svensson, B.; Rose, D. R.; Pinto, B. M. *Tetrahedron Assym.* **2005**, *16*, 1035-1046.

[132] Hiraizumi, S.; Ulrike, S.; Spiro, R. G. *J. Bio. Chem*. **1993**, *268*, 9927-9935.

[133] Spreitz, J.; Stütz, A. E. *Carbohydrate Research*, **2004**, *339*, 1823-1827.

[134] Belakhov, V.; Dovgolevsky, E.; Rabkin, E.; Shulami, S.; Shohamb, Y.; Baasova, T. *Carbohydr. Res.* **2004**, *339*, 385–392.

[135] Wu, X.; Tang, X.; Xian, M.; Brauschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem*. **2002**, *10*, 2303-2307.

[136] Voet, D.; Voet, J. G.; Pratt, C. W. Fundamentals of Biochemistry.

[137] Nelson, D. L.; Cox, M. M. Lehninger Principle of Biochemistry 3rd Edition

[138] Simon, M. S. *Cell Metabolism*, **2006**, *3*, 150-151.

[139] Berg, J. M.; Tymoczko, J. L.; Stryer, L. Biochemistry, 6th Eddition

[140] Seagroves, T.N.; Ryan, H.E.; Lu, H.; Wouters, B. G.; Knapp, M.; Thibault, P.; Laderoute, K.; Johnson, R. S. *Mol. Cell. Biol.* **2001**, *21*, 3436-3444.

[141] Kim, J.; Tchernyshyov, I.; Semenza, G. L.; Dang, C. V. *Cell Metab*. **2006**, *3*, 177–185.

[142] Sugden, M.C.; M. Holness, M.J. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E855–E862.

[143] Denko, N. C.; Lim, A. L.; Fontana, L.; Cairns, R. A.; Papandreou, I. *Cell Metabolism*, **2006**, *3*, 187-197.

[144] Fantin, V. R.; St-Pierre, J.; Leder, P. *Cancer Cell*, **2006**, *9*, 425-434.

[145] Bui, T.; Thompson. C. B. *Cancer Cell*, **2006**, *9*, 419-420.

[146] Kolb, H. C.; Finn, M. G.; Sharples, K. B. *Angew. Chem. Int. Ed*. **2001**, *40*, 2004-2021.

[147] Rostovtsev, V. V.; Sharpless, K. B.; Fokin, V. V.; Green, L. G. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.

[148] Tornøe, C. W.; Christensen, C.; Madal M. *J. Org. Chem*. **2002**, *67*, 3057-3064.

[149] Lee, L. V.; Mitchell, M. L.; Huang, S. J. Sharpless, K. B.; Fokin, V. V.; Wong, C. H. *J. Am. Chem. Soc.* **2003**, *125*, 9588-9589.

[150] Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164-11165.

[151] Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem. Int. Ed*. **2004**, *43*, 3928–3932.

[152] Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G.; *J. am. Chem. Soc*. **2005**, *125*, 3192-3193.

[153] Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. am. Chem. Soc*. **2005**, *127*, 210-216.

[154] Baldino, C. M.; Chane, P. C. M.; Isomo, Y.; Fokas, D.; Orgueira, H. A. *Tetrahedron Lett.* **2005**, *46*, 2911-2914.

[155] Chittaboina, S.; Xie, F.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 2331-2336.

[156] Bae, I.; Han, H.; Chang, S.; *J. Am. Chem. Soc*. **2005**, 1*27*, 2038-2039.

[157] Siemsem, P.; Livingston, R. C.; Diederich, F. *Angew. Chem. Int. Ed*. **2000**, *39*, 2632-2657.

[158] Xie, J.; Seto, C. T. *Bioorg. Med. Chem.* **2007**, *15*, 458–473.

[159] Ness, R. K.; Fletcher, H. G.; Hudson, C. S. *J. Am. Chem. Soc.*, **1950**, *72*, 2200.

[160] Fiandor, *J.*; Garcia-Lopez, M. T.; de Las Heras, F. G.; Mendez-Castrillon, P. P. *Synthesis*, **1985**, 1121.

[161] Prepared following literature procedure ref 68.

[162] We thank Prof. Vassella for providing the ezyme inhibition procedure.